

A Case for More Kidney Transplants

THE furore caused last week by the removal of the kidneys of a teenage boy without his parent's permission being obtained can be guaranteed to have at least one effect. And that is that surgeons, until the memory of the incident is erased, will find it even more difficult than usual to obtain kidneys for transplantation. The supply of kidneys in Britain for transplantation is nothing if not erratic and in spite of publicity and campaigns carried out by the Department of Health and Social Services, the last of which was launched six months ago, there is a serious shortage. Sadly, whenever a transplant operation, or the removal of kidneys from a cadaver as in this case, receives a great deal of publicity the supply gets even worse. The first reasons for this is that those who are asked to give permission for organs to be removed from deceased relatives from that they will receive undue publicity. The second is a more fundamental, but unfounded, fear that surgeons when given permission to remove an organ might proceed without being as certain as they should be that all signs of life had left the dying donor.

But what does the law allow? The removal of organs from bodies is governed by the Human Tissue Act of 1961 which, without prejudice to the case where the deceased has made an explicit decision to donate organs, provides that the person lawfully in possession of the body may authorize the removal of parts of it provided that—having made such reasonable enquiries as practicable—he has no reason to believe that there was objection on the part of the deceased before death or that the surviving relatives would object. That this is ambiguous, is to say the least. Who indeed is the person legally in possession of the body and how can “reasonable enquiry” be defined? These are the questions which a committee set up with Sir Hector MacLennan as chairman addressed itself to in 1969, but the committee failed to provide a unanimous recommendation for the way in which the law should be modified. Consequently the law remains ambiguous and incidents such as took place last week continue to make the lot of the transplant surgeon difficult.

But what are the options? There is a patent need for more kidneys for transplantation and on the face of it it seems that the best source of these is the 6,000 people who are killed on Britain's roads every year. The call in Britain at present is for some 2,000 kidneys a year but in fact only about 500 kidney transplants were carried out in 1972. In spite of the fact that the prognosis for patients with transplanted kidneys is getting better every year, barely 15% of those who need transplants benefit. The net result is that between 1,500 and 2,000 people between the ages of 15 and 55 die every year in Britain from malfunction of their kidneys. The situation is such that according to one estimate 90% of people who suffer from fatal kidney diseases die without treatment. An alternative treatment to transplantation is a dialysis machine but here the problems of cost and the avail-

ability of such machines rules against this being the universal solution. These are the facts which most unfortunately have not been given sufficient publicity in the past two weeks.

But before there are any large changes in the law—for example to allow organs to be transplanted unless the dead person is on a register of those unwilling to allow this—there must be a change in the public attitude to transplants. So what are the possibilities in the meantime?

The law at present is based on a contracting-in scheme and the question is should the present law be refined so that it would favour the surgeon? This would entail, for example, defining the person legally in charge of the body as the hospital or hospital authorities at the time of death and to interpret the phrase, “reasonable enquiry as may be practicable” as a commonsense statement that an attempt should be made to obtain the consent of relatives but that practicability should pay regard to the limited time available between death and when the kidneys must be removed. But such a proposal seems certain to run into heavy water if it is put before the public and Parliament.

The Department of Health and Social Service is committed to a campaign to persuade people to contract-in to a kidney donation scheme by filling in and carrying on them a card signifying that their organs can be removed in the event of their death. This scheme will not provide a large enough pool of donors to solve the problem but the hope of the department is that it will generate enough publicity to make the public aware of the need for kidneys. In this light it is not surprising to find that the names of potential donors are not recorded in a central registry. But if the DHSS is to demonstrate that it is serious about its intentions then the campaign to enrol potential donors should be stepped up and consideration given to the setting up of just such a central registry.

100 Years Ago



THE following are the principal additions to the Brighton Aquarium during the past week:—10 Thornback Rays (*Raja clavata*), 1 Large Tope (*Galeus canis*), 1 Large Smooth Hound (*Mustelus vulgaris*) 3 Three-bearded Rockling (*Motella tricolorata*), 1,000 Sticklebacks (*Gasterosteus spinosus*), 1 fine group of *Actinoloba dianthus* (orange variety); a Smooth Hound (*Mustelus vulgaris*) gave birth to seven young ones, which died immediately, or were born dead.

From *Nature* 8, 273, July 31, 1873



OLD WORLD

Medical Research Council Surfaces Intact

A SLIGHTLY grudging admission that things may turn out to be all right after all characterizes the Medical Research Council's first annual report since the government's white paper on a *Framework for Government Research and Development*, published last July (MRC *Annual Report*, HMSO, £0.90).

Staunchly defending the need for policy making in fundamental and applied research to be kept together, the council states that the Department of Health and Social Security has recognized this need. In its own inimitable verbosity the council declares that "good grounds exist for the hope that a system will develop within the new framework which will take full account of the problems to be faced and which will provide for coordination and collaboration between the departments and the council without detracting from the council's independence".

With a slight air of defiance which suits well the council that fought the Rothschild changes hardest, the MRC says that the white paper "may affect the practical policies that the Medical Research Council adopts". But it can neither "alter the inherent characteristics of scientific research" nor diminish the council's responsibility to advance medical science.

The council concedes that "there is a need to improve the planning of short-term research designed to answer questions of immediate significance" but warns that there must be no significant diversion of resources from longer term research to short term objectives "if the effects of such major afflictions as cancer, mental disorder and cardiovascular disease are to be moderated".

But once it has got past the flagwaving, the council explains that under the new organization four members of the council can be nominated by departments that have an interest in the council's work. The Secretary of State for Education and Science is to decide which departments name the four members. Meanwhile the council is reviewing its own board and committee structure, so that full details of precisely how the departments and the MRC plan to work together are not yet available. It has, however, been agreed that the departmental Health Services Research Board will act as an adviser to the council, while the council's committees and boards will advise the departments.

Turning from politics to science, the council states that arterial disease, environmental factors in disease, mental health and microbial disease are being kept under continuing review "because

of their social importance", while it wants to expand research on cancer, drug misuse and dependence, particularly in relation to smoking and alcoholism, and population problems, because "there is now both the need and the opportunity to take positive steps".

RESEARCH AND DEVELOPMENT

DTI tells all

EXPENDITURE on research and development by the Department of Trade and Industry is to increase by £9 million this year. In the first of its annual reports on its research and development activities, the DTI reveals that its expenditure on research and development was £57 million last year and is to rise to £66 million in 1973-74. The report also provides a detailed account of the working of the new requirements boards which were set up last year in the wake—although not at the instigation—of the government's white paper on research and development.

This new account of expenditure meets an undertaking given by the government to the Select Committee on Science and Technology that all departments would publish annual reports on their research and development activities.

The report emphasizes that the requirements boards purpose is first to identify those areas which will most benefit from government-supported R

The council also hopes that the closer involvement of government departments that will follow the white paper will allow for more clinical research.

The report also shows that the MRC's rate of expenditure is increasing ever more slowly. The transfers of funds to

and D, and second to determine the objectives and balance of research and development programmes to support departmental policies. "The initiative for new research may come from the boards themselves" the report says, "or from DTI customer divisions, or they may be bids for support from potential contractors".

Nine requirements boards have been established so far, and various special committees—some appointed on an *ad hoc* basis are to look after work that does not fall directly to any one of the boards—for example work at some of the research associations or at the Analytical Research and Development Unit at Harwell.

Contracts are placed with the DTI's own industrial research establishments, the UKAEA, the research associations, industrial organizations, the universities and research councils. The boards have also taken responsibility for the £1.69 million of work—on oceanographic sciences and universal resources—that the Department took over from the Natural Environment Research Council on April 1 under the changes outlined in the white paper.

Analysis of DTI Research and Development Gross Expenditure by Main Objectives

Objective	1972-73	£000*	1973-74
1 Nuclear R & D (safety)	74		165
4 Exploration and exploitation of the earth	685		996
4.1 Soil and substratum			
4.2 Seas and oceans	—		1,690†
5 Protection and promotion of health (e.g. control of pollutants)	1,976		1,859
6 Planning of environment	320		525
8 Promotion of industrial productivity and technology:			
8.0 General	4,884		5,113
8.1 Fuel (including electrical power)	75		122
8.2.1 Chemical	224		244
8.2.2 Metals	445		734
8.2.3 Electronics	1,087		1,652
8.2.4 Aerospace:			
Civil aeronautics	13,910		17,510
Space technology	9,055		13,930
8.2.5 Other transport	2,016		2,245
8.2.7 Mechanical engineering products	3,853		3,005
8.2.9 Miscellaneous products	2,203		2,321
8.9 Other research	1,345		1,633
9 Computer science and automation	12,425		8,749
Miscellaneous (grants to Research Associations, etc.)	3,140		4,183
	57,717		66,721

*The figures given for both 1972-73 and for 1973-74 are estimates.

†Work transferred from NERC on April 1 following last year's white paper *A Framework for Government Research and Development*.

government departments outlined in the white paper did not take place in the year the report covers, but it shows a decline in the growth rate to only 3.2% in real terms against a growth of 6 to 8% *per annum* four to five years ago. Total expenditure amounted to £28.5 million, £26.8 million from the parliamentary grant, the remainder coming from government departments, private bodies and other sources.

NUCLEAR POWER

New Company Attacked

A DAMP squib of a report on nuclear reactors is all that the Select Committee on Science and Technology has managed to produce after a lengthy spell of study.

Evidence accumulated from the United Kingdom Atomic Energy Authority, British Nuclear Design and Construction, the Nuclear Power Group, Sir Arnold Weinstock, Mr Tom Boardman, the Department of Trade and Industry, the Central Electricity Generating Board, and the Nuclear Installations Inspectorate has led the select committee to make six recommendations, some of which have been at least partially overtaken by events.

The committee recommends that

- unless the Nuclear Power Board is to be given an "effective role", it should not be appointed; in any case the new design and construction company should not be represented on the board;
- the government should take at least a 30% interest in the new company, and no single commercial interest should have a holding larger than 30%;
- the new nuclear company should hire its management expertise on a contract basis; (At present the government has given this role to GEC along with the 50% holding the company is to have);
- the CEBG should be required to order another nuclear power station very soon with government financial help if necessary;
- the development of the HTR should be expanded;
- the Chief Inspector of the Nuclear Installations Inspectorate should not hold any other post within the DTI.

This last point stems from the fact that Mr E. C. Williams, the chief inspector, told the select committee that he is also head of the Energy Technology Division of the DTI and as such gives technical advice to other sections of the department that deal with the fuel and power industries. The select committee states that the chief inspector should be clearly seen to be independent of policy considerations, and as such its recommendation seems sensible enough.

But the recommendation that the CEBG should order a new station shortly has already been met. It is clearly understood that a nuclear station is to be ordered next year when the government has decided which reactor type is to be built. Mr Arthur Palmer, chairman of the committee during the study, excused this by saying their report was based on evidence given by Mr Arthur Hawkins almost a year ago.

Equally the committee's recommendations about the structure of the new company arrive just after the structure has been decided, and the government is unlikely to chop and change at this stage. Does Mr Palmer think the committee's opinion will have any effect now? "Yes" said Mr Palmer, "there's a very good chance it will."

As for the recommendation that the Nuclear Power Board be given an "effective role", the committee fails to state what it considers such a role to be. It's only practical suggestion is that the board should have a full time chairman to "guarantee its key position in the scheme of things"

Having considered the thorny question of choosing the next reactor type, the committee concludes—in the wake of the departmental review carried out by Mr Peter Vinter of the DTI—that the choice is difficult. But the committee does appear to be withdrawing support for its long-time favourite, the steam generating heavy water reactor (SGHWR). The lack of enthusiasm for the system expressed by many of the witnesses—particularly with regard to the export potential—has finally damped the committee's fervour, and it recommends that the high temperature reactor (HTR) becomes a "major research and development effort" while "the government should undertake a serious appraisal of the work being done on the SGHWR; it might well be that there is little point in continuing it, sad as is such a conclusion after all the hopes of earlier years".

SCIENTIFIC RESPONSIBILITY

An Eye on the Future

SCIENTISTS are again to have their consciences stirred and their worries taken over by a public body. A Council for Science and Society, at the instigation of one Paul Sieghart, a lawyer, has appointed itself as a latter day, establishment-nurtured and charity-financed version of the British Society for Social Responsibility in Science.

At a meeting at the Royal Society last week, the council declared that it "will try to identify areas of research in science and technology which could have important social consequences for

good or ill, but which are not yet fully explored; to study these objectively, to attempt to foresee what their consequences might be; whether they should be controlled, and how; and to publish responsible reports designed to stimulate wide public debate about some of the issues of the future, based on the best information available. In this way it hopes to foster an active corporate social conscience in which the British scientific community can work".

And if you think you have heard those sentiments before, they differ little from the aims outlined in the BSSRS manifesto.

Not that the two organizations are the same. Paul Sieghart, who set up an interdisciplinary working party in 1971 to consider the problem of a corporate conscience for the scientific community (see *Nature*, **239**, 15; 1972), sees BSSRS as a complementary pressure group to the work of the new council which is to be a "credible establishment body, producing sober and thoughtful reports".

The council's membership is long, distinguished and scarcely lacking in heavyweight names, with Professor Sir Michael Swann as chairman.

The subjects that Sieghart envisages occupying the council are those "just over the horizon" rather than those actually with us. In other words, not Concorde or pollution, but rather mood control drugs and genetic engineering. But the council does not have a programme of work. Sieghart is hoping that the subjects that the council will consider will be suggested by scientists actually working in the fields in question. "We will welcome suggestions from anyone," he says. "We want the largest possible shopping list."

Once subjects have been identified the council will investigate them, point out possible dangers and highlight possible solutions, handing the results over for Parliament, the press and the public to mull over. Finance for the first three years of the council's existence is being provided by the Leverhulme Trust in the form of £80,000.

Why a new organization, though, to inform the public of the scientists' fears? Paul Sieghart says that "the institutional machinery available to scientists for the most effective performance of their special social obligations is at present quite inadequate". The Royal Society, Mr Sieghart says, has not performed the role of social conscience "to the full extent necessary in modern times", and the British Association has, at present, neither the money nor the organization. But there are many who will wonder whether such an "establishment" organization might not have best been created within the existing frameworks.

NEW WORLD

Hopes and Fears for US Science Policy

by our Washington Correspondent

THIS month marks the end of an era in US science policy and the beginning of another. That, at least, is the theory, for on July 1 the Office of Science and Technology was formally abolished and its functions transferred from the White House to Dr H. Guyford Stever, Director of the National Science Foundation. The machinery established by Presidents Eisenhower and Kennedy, which gave science a place in the highest corridors of power in the United States, has thus been dismantled and the head of a tiny government agency—described by Dr Stever himself as “the junior partner in science”—has been given the task of watching over his big brothers in the rest of the federal establishment.

But is the new machinery really so different? Last week the House Committee on Science and Astronautics held two days of hearings to discover how the new science policy apparatus may work in practice, and it became clear that in the six months which elapsed between OST's sentence and execution, new arrangements evolved which, in a few key respects, bear a striking resemblance to the old. Nevertheless, suspicions that science has been downgraded in the federal government have not been entirely allayed.

When the new arrangements were announced in January, there was strong criticism of the fact that Dr Stever would be Science Adviser not to the President, but to the President's assistants. Unlike Dr Edward E. David who, until he resigned in January, bore the title Science Adviser to the President, Dr Stever's official title is Science Adviser, but to nobody in particular. The original idea was that his advice would go chiefly to Dr George Shultz, Secretary of the Treasury and President Nixon's special assistant on economic affairs. But last week Dr Stever made public a letter he received from the President on July 1, in which Nixon formally designated him as “my Science Adviser”.

It may be merely a semantic difference, but members of the committee were eager to know the significance. Stever said, in reply to a question from Mr Ken Hechler of West Virginia, that the letter clarifies his responsibilities “quite a bit”, and that he feels he “can go to the President on a matter of urgency”. He later said, however, that he would expect to take Shultz with him on such a visit. How many times

has Stever met the President? Three times since January, once for a lengthy meeting on international scientific relations, and two “much shorter meetings on other matters”, Stever replied, and added that he has had “more frequent conversations with Mr Ash (Director of the Office of Management and Budget) and Mr Shultz”.

Three visits to the President in six months does not suggest a close relationship between Mr Nixon and his Science Adviser, but as one former OST staff member pointed out last week, “none of the committee members asked how many times Dr Edward David saw the President”. He suggested that Stever's record of visits is not bad in comparison, but that a more important question is whether or not advice reaches the President through channels other than personal representation. One of the hallmarks of the Haldeman-Ehrlichman era at the White House, he said, was that advice, memoranda and reports from the OST were ignored or buried and seldom reached the President's attention. The post-Watergate White House may be more receptive to scientific advice.

Perhaps more important than the number of audiences Mr Nixon grants to his Science Adviser is the working relationships between science policy-makers and other elements of the White House, particularly the Office of Management and Budget. Since it involves the lifeblood of the federal agencies, preparation of the budget estimates of the Administration is the most important annual exercise in the executive branch of the government, and the OST's chief influence came from day-to-day workings with officials of the OMB. Have those contacts been maintained now that science policy advice has been shifted from the White House?

Dr Stever announced earlier this month that he has established a small Science and Technology Policy Office (STPO) to assist him in his new role (see *Nature*, 244, 5; 1973), and it is this office which will carry out most of the work with OMB. It so happens that the ten staff members, including secretaries, so far appointed to STPO are all former staff members of OST, and they are also located in the old OST offices in the Executive Office Building. At the working level, the new arrangement thus looks very much like a slimmed down version of the old one.

One point on which committee members expressed considerable reservation

is that Dr Stever must wear two very different hats in his duties as Director of the National Science Foundation and as Science Adviser. He may, for example, face a conflict of interest in advising OMB how to divide the pie for science and technology among the various agencies of the federal government. Stever agreed that his dual responsibility is a potential problem, but said several times during his testimony that STPO has been deliberately set up outside the mainstream of usual NSF activities to provide an independent source of advice to him.

In April this year Dr Stever established in the National Science Foundation an Energy Research and Development Task Force, under the direction of Dr Paul F. Donovan, to provide advice to him on energy policy. But, with the appointment of Mr John Love as President Nixon's special adviser on energy policy, and the establishment of the Energy Policy Office in the White House (see *Nature*, 244, 4; 1973), the functions of the NSF's task force have to some extent been overtaken. Nevertheless, Dr Stever said last week that “one of the principal objectives” of the task force will be “to contribute to the formulation of the 1975 budget”. It did not escape the committee's attention that, after abolishing the Office of Science and Technology, President Nixon has established the Office of Energy Policy in the White House to carry on one of the OST's functions.

It thus seems that the new science policy machinery evolving under the Director of the National Science Foundation is, in some respects, not very different from the old regime. But there are, however, two important changes to be noted.

The first is that the President's Science Advisory Committee, which provided advice to the White House, has not been reinstated. Dr Stever said last week that he has not yet made up his mind about how best to seek the views of the scientific community, but stated his “personal philosophy that advisory groups are best used on an *ad hoc* basis, selected for a particular problem, and kept in operation only for the life of the problem”. The other change is that Dr Stever has no brief for giving advice on defence research and development.

It so happens, however, that the Department of Defense receives about half of all federal research and development funds.

ALASKA PIPELINE

Confusion and Irony

by our Washington Correspondent

THE Senate last week passed a remarkable piece of legislation. It voted to clear away all legal obstacles to construction of the trans-Alaska pipeline, and turned down an amendment which would have delayed a start on the venture until an alternative route through Canada had been given detailed review. The vote and debate were surrounded by irony, for the sponsor of the legislation is one of the staunchest supporters of some environmentalist causes in the Senate, and it was passed over the opposition of one of the staunchest supporters of the pipeline. Moreover, it was passed only after Spiro Agnew had, for the second time in his career as Vice-President, used his casting vote. But the most ironic aspect of the affair is that, according to some observers, the legislation raises constitutional issues that could delay construction of the pipeline for another two years.

The trans-Alaska pipeline has been held up by legal challenge from environmentalist groups who have argued that the Department of Interior has not fulfilled the dictates of the National Environmental Policy Act (NEPA) in granting a right of way for the pipeline. But in February this year, an appeals court in Washington DC astonished supporters of the venture with a ruling that, whatever the merits of the environmental argument, the proposed right of way for the pipeline is wider than a 1920 statute allows. For the past two weeks the Senate has been busy repealing that ancient law, and the key vote came on an amendment sponsored by Mike Gravel, a Senator from Alaska. The amendment, in short, clears away not only the right of way technicality, but all environmental challenges as well.

When the appeals court handed down its ruling on the right of way matter, it specifically declined to offer an opinion at that time on the environmental question raised by opponents of the pipeline. But Senator Gravel's amendment states that it is the opinion of Congress that the Administration has complied with NEPA, and in the words of Senator Ted Stevens, Gravel's colleague from Alaska and a co-sponsor of the amendment, "we are in fact trying to get Congress to substitute its judgment for the judgment of the court". If the amendment is supported by the House of Representatives, the effect will be to blast away all further legal challenge to the project. At least, that is what the sponsors are hoping.

But their interpretation is open to question, and in the opinion of some,

it could have just the opposite effect. One such doubter is Senator Henry M. Jackson, one of the most vocal supporters of the trans-Alaska pipeline but also the chief author of NEPA. In the debate on the amendment, Jackson said that he believes it "invites delay by creating numerous new opportunities for litigation".

First, he argues that the amendment does not exempt the pipeline from the requirements of NEPA because even if Congress states its opinion that the Administration has followed the dictates of the law, the courts will wish to make an independent judgment on the matter. It will take at least two years, he suggests, to decide whether or not the amendment does in fact exempt the pipeline from further NEPA review. Second, Jackson argues that the amendment invites a legal challenge on the constitutional question of whether or not Congress has the right to usurp the power of the courts. Third, Jackson is concerned about the precedent of by-passing NEPA: does Senator Gravel, "who has often expressed concern over the hazards of nuclear power want to exempt the breeder reactor program from NEPA and judicial review", he asked.

Senator Stevens replied, however, that the amendment has been looked at by constitutional lawyers, and that there is ample precedent for Congress issuing findings of fact and then denying the right of the courts to review those findings. But the most compelling argument for clearing away legal barriers to the pipeline turned out to be the energy crisis, which kept popping up throughout the two weeks of debate on the right of way matter, and in the event, the amendment was passed by 49 votes to 48. There was then a motion to reconsider the vote, which was split evenly 49 votes to 49, and Mr Agnew used his casting vote to enable the amendment to stand.

The focus has now turned to the House of Representatives, where similar legislation was being considered last week by the Interior Committee.

There is, however, another alternative waiting in the wings. Jackson said last week that if the pipeline is held up any further by litigation, particularly in regard to yet another factor—a disagreement between the oil companies and the State of Alaska regarding right of way across State land, which is at present being fought in the State courts—he will introduce legislation for the Federal Government to take over construction and operation of the pipeline. In other words, the pipeline would be nationalized, and that, in the words of a lawyer involved in the litigation, "would open a whole new can of worms".

APPOINTMENTS

New FDA Chief

CASPAR WEINBERGER, Secretary of Health, Education and Welfare, confirmed last week that Dr Alexander MacKay Schmidt will be the new Commissioner of the Food and Drug Administration. Dr Schmidt, who for the past two and a half years has been Dean of the Abraham Lincoln School of Medicine at the University of Illinois, has been tipped as the new FDA Commissioner for several weeks. He succeeds Dr Charles C. Edwards who was promoted earlier this year to the post of Assistant Secretary for Health in HEW. Born in 1926, Schmidt received his BS degree from Northwestern University in 1951 and his MD degree from the University of Utah in 1955. As Commissioner of FDA, Schmidt will occupy one of the hottest seats in the federal government, for the FDA is constantly caught in the crossfire between food and drug manufacturers and skilful and increasingly vocal consumer groups.

BUDGETS

Nixon's Warning

by our Washington Correspondent

ONE item in President Nixon's statement on Phase 4 of his economic game plan, announced last week, holds important implications for the scientific community. Mr Nixon announced that because "confidence in our management of our fiscal affairs is low, at home and abroad", the Administration has adopted the goal of balancing the federal budget. "It is clear," President Nixon said, "that several billion dollars will have to be cut from the expenditures that are already probable if we are to balance the budget". And he also said that he would instruct federal departments to reduce their payrolls below the levels budgeted for the 1974 fiscal year.

The announcement is important to science because the share of the federal budget devoted to science and technology is particularly vulnerable to cut-backs. About two thirds of federal expenditures cannot be cut because they go to such items as welfare, social security and pensions, for which prior commitments have been made; the cuttable third includes the entire science budget. It will be remembered that last year, when faced with the prospect of either increasing taxes or reducing federal expenditure, President Nixon opted for the latter course, cut back heavily on appropriated funds and vetoed several appropriations bills. Particularly hard hit were the Department of Health, Education and Welfare (HEW), and the National Institutes of Health.

NEWS AND VIEWS

Programs for Learning to Think

IF Dewey was right that we learn by doing, and by thinking about what we do, then the goals of educational innovation are clear. We must invent better things to do in educational establishments and better ways of thinking about ourselves doing them. One aspect of current work in artificial intelligence which was not mentioned in the Science Research Council's recent Lighthill Report on the subject (see *Nature*, **243**, 318; 1973) is the role of computers in school education. The possibility that children of the ages of about ten and upwards could learn to program computers to control toy vehicles, draw geometrical figures, and compose music or poetry is at the heart of the LOGO project, which is Seymour Papert's response to Dewey's ideas.

Papert's writings on the subject are rather scattered, and not always easily available, though they can be obtained from the Artificial Intelligence Laboratory at MIT, and some have been published, for example in *Mathematics Teaching* (No. 58, 2; 1972) and in the proceedings of the NUFFIC Summer School, *Process Models for Psychology* (edited by D. J. Dalenoort, 1; Rotterdam University Press, Rotterdam, 1973). The essential features of the teaching project are that children in an ordinary American grade school have been introduced to computing by learning a simple language, LOGO, which they can use to control a turtle-shaped vehicle, with a retractable pen to draw on the surface on which it runs. Alternatively, they can draw or write text on a visual display, or control a bank of resonators to make music, or indeed operate any other device of an electromechanical kind.

Part of a typical beginning turtle program might, for instance, be a procedure to draw a triangle, as follows:

```
TO TRI :SIDE      1. FORWARD :SIDE
2. RIGHT 120      3. TRI :SIDE
```

Here the numbered lines are instruction statements, for example line number 2 instructs the turtle to turn clockwise through 120 degrees. In statement 1, FORWARD is an instruction to move forward. The colon indicates that what follows is not a literal but a name, in this case of a variable indicating the number of units to move. At the head is the name of the procedure, TRI, prefaced by TO which indicates that what follows is a definition, that is in this case how to draw a triangle. In the example, if a value of the procedure parameter SIDE is made available, for instance, by calling the procedure with the statement TRI 100 then the turtle will continually trace an equilateral triangle of side 100 units, by re-entering the procedure recursively from statement number 3.

The notion of a recursion in which a process is defined partly in terms of itself is well known to be difficult even for adults. In this project, though, children of a tender age come to grasp it not by having been instructed in the arcane hieroglyphs of algebra, but by controlling a toy vehicle. By starting with intuitions and concepts of movement and space which are real and immediate to

children, and by giving them a symbolism that relates as closely as possible to these intuitions but which is nevertheless formal, one can introduce them to powerful ways of designing processes. In a sense the processes children design are rather like essays. The difference is that in LOGO not only can they extend the language themselves, but when they write an essay it is actually executed by the machine. This leads not only to a deeper understanding of the logical consequences of what they write, but opens up new worlds of surprising discoveries. For example, writing as a child might, FORWARD :SIDE +1 as statement 1 in the TRI procedure creates a spiral triangle. Children can thus gain an understanding of notions such as length and angle from the interplay between the symbols they generate and the real world, but they can soon experiment with the more complex ideas of variable, vector, state, and even quite advanced matters such as procedures and recursion, and in doing so make discoveries about the meaning and power of these ideas.

The point of starting with a compatible and immediate symbolism as a means for thinking about the world is important enough. But perhaps even more important is that in doing this children extend their own thought processes. In particular as they make mistakes, the logical consequences of the programs they type into the computer are demonstrated to them in the behaviour of the device they are trying to control. No teacher writes 0/10 in embarrassing red pencil on their exercise book, or tells them they have failed. In trying to draw a triangle, for instance, they might write RIGHT 60, and thus produce a figure which is not a triangle but a hexagon. They project such mistakes onto the program itself, and set about debugging it. In order to do this they necessarily have to acquire a deeper understanding of the process they are trying to create. Thus the gap between the desire and the fulfilment is bridged by the children debugging their own thought processes and coming themselves to grasp fundamental concepts of logic and process.

Although the project has produced no statistical comparisons with more conventional teaching methods, and any scholastic improvements one might see may be due to Papert's engaging personality and his interest in the children, it is difficult to argue against the need to invent new ways of thinking about the world if intellectual progress is to be made; and work in artificial intelligence has gone a long way in capturing the art and science of formally describing and using knowledge. The LOGO project is grounded in the understandings of epistemology that have emerged from the MIT Artificial Intelligence Laboratory, and starts from the idea that children as well as adults can benefit from expressing knowledge formally. The knowledge in their programs is applied to the design of processes which will do things that interest them, and the interplay between the symbolism and its execution provides not only for discovery but for thinking about how the processes work, and for thinking about thinking.

from our Experimental Psychology Correspondent

T Cells Suppress T Cells

WHAT shall we find in the pot at the end of the immunological rainbow? There will be T and B lymphocytes and their various subpopulations with a list of their differing properties and roles in immunity, a chemical wiring-diagram outlining the mechanisms involved in signalling the cells to "turn on" or "turn off" on encounter with antigen, and a map of the interactions between T and B cells and macrophages, with footnotes on how these operate.

That humoral antibody produced by B cells can specifically enhance or inhibit (depending on the circumstances) the responses of T cells and other B cells seems well established, and the notion that T cells specifically help B cells to make antibody is now part of immunological dogma. It has been demonstrated recently that T cells can suppress (specifically and nonspecifically) the response of B cells. Although the modulating activity of T cells on other T cells has been less actively studied, there is now compelling evidence that two subpopulations of T cells can act synergistically and specifically to produce a graft-versus-host response, and there is one report of thymus cells inhibiting the response of other thymus cells.

In this issue of *Nature* (page 227), Zembala and Asherson fill in what may be the final arrow in the inter-relationship diagram, by demonstrating that peripheral T cells can suppress the response of other peripheral T cells. They have already shown that contact sensitivity to picryl chloride in mice (measured by ear swelling) is T cell dependent and can be suppressed specifically by pretreatment with picryl sulphonic acid. They have also shown that the suppression can be transferred by injecting lymphocytes from picryl sulphonic acid treated mice into normal recipients. Now they demonstrate that the suppressive activity of these cells can be abrogated by treatment with anti- θ serum and complement, which has been shown to kill T lymphocytes selectively. Although this establishes that the suppression is T cell dependent, it could operate through antibody secreted by B cells with T cell help. To exclude this possibility, Zembala and Asherson went on to show that suppressor cells can be generated by injecting normal thymus cells into lethally irradiated, syngeneic mice, which are then treated with picryl sulphonic acid, and the suppressor cells harvested from lymph nodes or spleen five days later. Although this strongly suggests that B cells are not involved in the suppression, one cannot exclude the participation of a small number of B cells contaminating the thymus inoculum or remaining in the irradiated host's spleen. More important, these experiments leave open the possibility that the T cell suppressor activity is indirect and mediated by a third party cell, such as a macrophage.

Thus it seems that T cells can specifically enhance and suppress the response of other T cells, just as they can B cells, but the mechanisms involved are unknown. In the case of T-B collaboration in humoral antibody responses it is known that the cooperative event(s) requires an antigen bridge between T cell receptors recognizing one determinant on the antigen and B cell receptors recognizing a different determinant on the same antigen. It is not clear whether T-T cell synergy or T cell suppressive effects have a similar requirement. It also

remains to be determined whether T cell helping and suppression functions are the properties of the same or different subpopulations of T lymphocytes. M. C. R.

Earthquakes on the Move

To the earth scientist, in recent years earthquakes have served as valuable markers of plate boundaries—regions where adjacent plates are in vigorous contact with each other. Typical velocities of relative motion are 1–10 cm yr⁻¹. Within the framework of plate tectonics there is a clear understanding of why they occur where they do, but the time element of earthquake occurrence is still very obscure. Some simple things can be understood. If strain has been unrelieved at a plate boundary for many tens of years then earthquakes are bound to ensue eventually, but there are no clues to exactly when simply from looking at past seismic history. On the other hand, it is also obvious that a really large earthquake will not be followed by another big one at the same spot for several years. But between these extreme and obvious statements lie the vast majority of quakes, the temporal pattern of which remains to be understood.

Of course, there may be no pattern to it at all. The process of initiating earthquakes could be entirely random, or at least too inscrutable for our present eyes. It could be related to extraterrestrial sources (such as tide generation) but a lot of work on periodicity has yielded nothing very convincing. What remains an attractive prospect for research is that in some sense earthquakes cause earthquakes. For example, a combination of elastic waves emerges from every quake and essentially redistributes strain throughout the whole Earth, although the strain changes at great distance may be one part in a billion or less. All parts of the Earth's surface are already under some strain and in places this may be near breakdown values, thus the passage of the elastic waves could temporarily raise the strain to breakdown. On the other hand the permanent strain change might bring the level somewhat closer to breakdown, increasing the long term probability of there being an earthquake.

One idea that has been gaining ground recently, however, is the hypothesis that seismicity migrates along faults with a speed somewhere between that of continent drift (cm yr⁻¹) and material rupture (km s⁻¹). In today's *Nature* (page 213) is a paper by M. D. Wood and S. S. Allen which brings some evidence to bear on this question of migration. A particularly active section of the San Andreas Fault south of San Francisco has been considered for a time span of forty years. There is a rather striking silence at the northern end of the section whilst earthquakes were occurring in the south in the 1930s. With time the activity moved to the north, and if the concept of migration velocity is a useful one, then a value of 3.3 km yr⁻¹ could be assigned.

It is tempting to predict the time and location of the next earthquake, and Wood and Allen chance their arm that there will be a moderate sized event within the next few years north of the recent Bear Valley activity. This region of northern California, highly populated with seismologists and seismometers, is proving to be quite a testing ground for prediction. W. Ellsworth and R. Wesson have already predicted a location for a smaller earthquake using very different techniques. D. D.

ECOLOGY

Prey and Predation

from our Animal Ecology Correspondent

ECOLOGISTS interested in interactions of predators and their prey have for long taken Volterra's classical work (*Mem. Acad. naz. Lincei* (ser. 6), 2, 31; 1926) as the starting point of their deliberations. There are, however, some aspects of this work that are far from satisfactory from a biological viewpoint. The chief amongst these, when both predator and prey are warm-blooded, is that the differential equation used assumes (1) that both predator and prey breed continuously and that the rate of breeding is directly related to the amount of food eaten, (2) that the rate at which prey animals are eaten is proportional to the probability of a chance encounter between predator and prey, and (3) that there is no other limitation on the prey population. It is hardly conceivable that these conditions operate in a situation in which there are sharply defined breeding seasons controlled by environmental factors and in which there is some time lag between the consumption of prey by a predator and the production of young. Lastly, a limit must eventually be imposed on prey populations although they may be kept below this asymptote by the activities of their predators.

J. Maynard Smith and M. Slatkin have developed a model to describe the interaction of vertebrate predator and vertebrate prey that goes much further than Volterra's (*Ecology*, 54, 384; 1973). They take as their starting point the age-old observation that predator-prey interactions are remarkably stable and populations of both coexist for long periods of time. Two stabilizing processes seem possible. The first is the movement of predators and prey in space, and the second is that owing to age and other differences, not all individual predators will be equally efficient hunters. In many species, especially of mammalian predators, the juveniles have to be taught how to hunt and when they are independent may concentrate on older or younger prey than do their parents. The new model examines the second of these possibilities.

The authors show by their computations that when stable coexistence occurs, the population of a prey species is only a little lower than the level at which it would achieve equilibrium in the absence of predators. If the prey population falls substantially below the environmental carrying capacity, instability follows and the extinction of one population results. Stable coexistence is enhanced by the variety of hunting ability found in mixed aged populations. In years when prey popula-

tions are low at the start of winter, survival of adult predators is good at the expense of juveniles. The Volterra model suggests that the presence of cover may increase stability but it seems from the new model that this is not so. Because the predators cannot hunt efficiently enough to annihilate the prey totally, cover for the prey will only make it more likely that the predators will become extinct.

The principal merit of the new model is that it regards the interaction from a sound biological viewpoint and attributes biological characteristics to the participating species and explains some of the evolutionary mechanisms resulting in the "prudence" attributed to predators when they do not over-exploit their food resources.

FOOD CHEMISTRY

Slimmer's Protein

from our Molecular Biology Correspondent

DEPENDING on one's outlook, the word protein may bring to mind a schlieren boundary or a beefsteak, provoke cerebration or salivation. In the bright light of modern protein chemistry the two images may now perhaps be united. It was never of course supposed that the blend of denatured muscle proteins confers the flavour on the beefsteak, for it is small molecules that have invariably been associated with taste. Indeed receptors have been isolated from taste organs, which will specifically bind sweet-tasting sugars, for example, but not others. Many amino acids, particularly D-isomers, are sweet, and at least one intensely sweet dipeptide has been discovered. The first example of a macromolecule with taste activity was the protein, miraculin, which has the remarkable property of transforming the sourness of acids into a sweet sensation. A protein which is sweet in its own right has been isolated and characterized in two laboratories in recent months. An earlier report suggested that it might be a glycoprotein, but this proved to be erroneous.

The protein, which comes from a tropical fruit, was isolated more or less pure by van der Wel (*FEBS Lett.*, 21, 88; 1972) by a two-stage gel filtration of a water extract, and later by ion-exchange (van der Wel and Loeve, *ibid.*, 29, 181; 1973). The sweetness was found to reside in the principal electrophoretic zone in a starch gel (prudently preferred for the purpose to polyacrylamide), scanning by tongue being apparently the method of detection. The molecular weight was estimated from the elution volume on the gel-filtration column as some 10,000. Morris and Cagan (*Biochim. biophys. Acta*, 261, 114; 1972) covered much the same ground using ion-exchange chromato-

graphy to prepare the protein, and pre-empted for it the name of monellin. More recently (Morris *et al.*, *J. biol. Chem.*, 248, 534; 1973) they characterized the protein in some detail, and found a molecular weight of 10,500 by SDS-gel electrophoresis and a similar value based on the minimal tryptophan content. The protein is rather basic, and there is one thiol and no disulphide bonds. More particularly van der Wel and Morris *et al.* are agreed that there is no carbohydrate. Proteolysis destroyed the flavour.

Last year van der Wel and Loeve reported the isolation of yet another sweet protein from a different type of fruit. They dubbed it thaumatin after the name of the plant, and found that it comprised two electrophoretic species, one of which appeared to be a partially deaminated form of the other. Again there is no saccharide. The molecular weight by sedimentation equilibrium was 21,000. The thaumatins are basic and contain seven disulphide bonds, in which respect they differ sharply from monellin. The nature of these proteins is an engrossing subject, and Korver, van Gorkom and van der Wel (*Eur. J. Biochem.*, 35, 554; 1973) have embarked on a conformational study. The circular dichroism shows a set of small Cotton effects arising from the tyrosine and tryptophan residues, and stronger features that can be assigned to the abundant disulphide chromophore. (It might be remarked that for the interpretation of disulphide circular dichroism in terms of the chirality of the torsional angle it is by no means sufficient to follow the precepts offered by Urry and his co-workers, in a spirit of optimism rather than rigour; one can conclude only that they are in a locked configuration.) There is clearly no appreciable content of α -helix. High resolution proton magnetic resonance spectra show high-field features characteristic of aliphatic protons perturbed by ring-currents of neighbouring aromatic side chains. These characteristics, together with a sharp thermal melting profile, the denaturation temperature depending on pH, which is manifested in a change of the circular dichroism and the generation of an ultraviolet difference spectrum, make it clear that thaumatin is a genuine globular protein. Its thermal denaturation, which is reversible, is accompanied by the disappearance of the sweet taste.

It thus seems that the taste is a characteristic of the native conformation, that is to say of a defined geometric constellation of residues. Both thaumatin and monellin exhibit sweetness to a prodigious degree, compared for example with sugars, and the sensation persists on the tongue for long periods. Now it is known that sugars bind only rather weakly to the taste

receptors and one may surmise that the sweet proteins associate with them much more strongly, in the manner of an antigen with its antibody for example. The search for gastronomically interesting proteins has now presumably been joined in earnest and no doubt the minds of workers in the field are busy with the possibilities of measuring the interactions of the sweet proteins with receptors, and of anchoring them to matrices, so as to capture receptor proteins by affinity chromatography. Cagan (*Science*, **181**, 32; 1973) has just reviewed the properties of miraculin and the two sweet proteins.

It might be noted that the isolation of a sugar-binding receptor protein was reported last week by Hiji and Sato (*Nature new Biol.*, **244**, 91; 1973).

PEST CONTROL

More Protein for Africa

from a Correspondent

UNDER the auspices of the Department of Agriculture of the Rockefeller Foundation, twenty of the chief workers on tsetse flies and trypanosomes from many countries recently gathered at the magnificent Villa Serbelloni on the shores of Lake Como. The purpose of the gathering was to scrutinize the relationships between tsetse flies and the pathogenic trypanosomes which they transmit to man and domestic animals in Africa and in particular to make some progress in deciding the most promising approach to the control of cattle trypanosomiasis—a disease which denies large areas of Africa to cattle, seriously reducing the amount of animal protein produced in that vast continent.

The new research data were related during the numerous stimulating discussions to the control of trypanosomes by chemotherapy or by immunization of cattle and to the control of tsetse flies by insecticides or by biological methods. The main difficulty with chemotherapy on a large scale is that of cost and practicality, because of the repeated dosing that is necessary with available drugs. Pharmaceutical companies are reluctant to invest in developing more effective and more easily applied trypanocides. Immunization of calves against trypanosomes would be an ideal alternative to chemotherapy, if only long lasting immunity could be guaranteed. Unfortunately the antigenic properties of trypanosomes are extremely plastic for reasons that are imperfectly understood. Much work is needed on the whole subject of variable antigenicity, though this will only be possible once large quantities of fly-adapted trypanosomes are available, and in this connexion, development of tsetse fly tissue culture techniques for growing the infective invertebrate forms of trypano-

somes could become of great significance in the future.

Traditional control methods of clearing natural vegetation and shooting game animals, together with the application of insecticides, were only briefly discussed. These three still remain the only effective methods of eliminating tsetse flies and controlling trypanosomiasis. They are expensive techniques which often need to be repeated and they are all ecologically unsound in some respects. Great hope was held out for the wide range of potential biological control methods discussed, some of which are urgently in need of further detailed study. For example, flies of the genus *Thyridanthrax* are commonly occurring parasites of tsetse in the wild; if only these parasites could be bred in captivity, then large numbers might be released in the field. Again, the midgut symbionts of the tsetse fly supply vitamins and when these symbionts are killed the flies soon follow suit, clearly suggesting a novel control method based on the destruction of tsetse symbionts with antibiotics introduced in the fly's diet. Another possible biological control method lies in blocking the anticoagulant of tsetse fly saliva, which would cause blood to coagulate in the fly's food canal and so prevent further feeding. These together with many other aspects of tsetse fly sensory, neural and energy metabolism would be fertile ground for pharmacologists with an interest in developing effective blocking compounds. This is a field which is virtually untouched at present.

The most advanced biological control method is based on the release of sterilized male tsetse flies, which mate with the wild female flies resulting in a marked decrease in numbers. This technique has been used with varying amounts of success on several other

types of flies. The tsetse is particularly susceptible to the sterile male technique because of its very slow rate of population increase, and if this technique was applied to a population of flies already much reduced in numbers by some other method, then success would seem certain. A prevalent opinion among the conferees was that different combinations of various control techniques would be needed to eliminate tsetse from different environments. It is clear that in each new control situation competent ecological advice should be sought prior to the implementation of the control programme. It is also of some importance that adequate economic, political and sociological information be obtained in advance so that the land freed for ranching is used to best advantage. The convivial atmosphere at the Villa Serbelloni encouraged a most open and relaxed exchange of ideas, to the mutual benefit of all of the participants and, ultimately, to the detriment of the tsetse flies and trypanosomes that plague the African continent.

ENZYMES

Evolution in the Test-tube

from our Molecular Genetics Correspondent

WHAT recourse has a bacterium when it irretrievably loses the function of one of its genes? One path open to the cell is the alteration of some other gene so as to code for a protein which can substitute for the lost function. Forced evolution of this nature has been followed in a series of experiments of remarkable elegance by Campbell, Lengyel and Langridge (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 1841; 1973).

The strain of bacteria used to provide the raw material for evolution carried a deletion in the *z* gene of the lactose

New Light on Myofilaments

ANTIBODIES to myosin have been used to study the disposition of myosin molecules in the thick filaments of muscle. It has recently been found that the presence of antibodies to another thick-filament protein, the C-protein, in the anti-myosin introduced some ambiguity into these results. With the isolation of C-protein, it became possible to prepare antibodies against it alone, and use them to identify its location in glycerinated muscle fibres. The results of these experiments are reported by Rome *et al.* in next week's *Nature New Biology*. Using X-ray diffraction, they found that labelling with antibodies to C-protein resulted in a large enhancement of a reflexion at 442 Å. The diffraction data, together with electron microscopy, are tentatively interpreted in terms of a periodicity from the C-protein equal to

that of the myosin. In an accompanying paper Rome *et al.* use the same approach to examine the distribution of the calcium-binding component of troponin, which is known to reside in the thin filaments. An X-ray reflexion from whole muscle on the meridian at 385 Å has been assigned to the I-band repeat, but its identification with troponin has been speculative. The calcium-binding component of troponin (troponin-C) was used to make antibodies and these were diffused into glycerinated muscle. The result was a large enhancement of the 385 Å reflexion, which as measured appears actually to be closer to 383 Å. This value is in better accord with the suggestions that the troponin repeat is seven times that of the actin subunits in the thin-filament helix.

operon; these cells therefore lacked the enzyme β galactosidase and so could not hydrolyse lactose to galactose and glucose (the principal reaction of the enzyme). The loss of β galactosidase is the only deficiency in the capacity to metabolize lactose of these cells; so the mutants should be able to regain the ability to utilize lactose by producing this single enzymatic activity.

The ability of cells to grow on lactose can be tested by plating them on Tet-lac medium, which contains the tetrazolium-lactose indicator. Cells unable to metabolize lactose form deep red colonies; but cells which can utilize the sugar grow white in colour. Another colorimetric reaction can be used to follow the activity of β galactosidase in extracts of the cell; the enzyme releases *o*-nitrophenol from the reagent *o*-nitrophenol- β -galactoside in a reaction which can be followed spectrophotometrically by its development of a yellow colour.

When placed on Tet-lac plates, the deletion strain of *Escherichia coli* at first formed only red colonies. But about one month later white colonies began to grow. The white colony which grew most rapidly, representing the cells with the most effective ability to hydrolyse lactose, was isolated and then transferred to a fresh plate containing minimal medium supplemented with the lactose. These *ebg-1* cells, so called for evolved β -galactosidase activity, then gave rise to several colonies, the fastest growing of which was isolated and replated as the *ebg-2* cell line. After successive cycles of selection, the *ebg-5* cells were obtained.

This cell line grows as rapidly as wild type cells on the lactose minimal agar and produces pink colonies on Tet-lac plates, compared with the red of *lac⁻* cells and the white of *lac⁺* cells. Extracts possess a lactose hydrolysing activity which releases *o*-nitrophenol more rapidly than extracts of *ebg-1*—a visual demonstration of evolutionary improvement—but more slowly than extracts of wild type *z⁺* cells which possess the enzyme β galactosidase of the lactose operon.

The enzyme responsible for the recovery of β -galactosidase activity in the *ebg-5* cells differs both physically and in the conditions for optimum activity from the enzyme specified by the *z⁺* gene of the lactose operon. The *ebg-5* extracts contain an enzyme which is even larger than β galactosidase (itself unusually large with a sedimentation coefficient of 16S and a molecular weight of 5.4×10^5), sedimenting at 24.4S with a molecular weight of about a million. The *ebg-5* enzyme is extremely sensitive to inhibition by ammonium ions, has a much sharper pH optimum than β galactosidase, and a K_m for *o*-nitrophenol- β -galactoside four times greater. Immunological tests confirm the conclu-

sion that the two enzymes are completely unrelated.

That five cycles of selection were used to isolate *ebg-5* implies that several different mutations must have contributed to the evolutionary revival of ability to hydrolyse lactose. The *ebg-5* characteristic behaves as a single gene in genetic crosses, implying that all these mutations must be located close together, presumably in the amino acid sequence of a single protein. The *ebg-5* locus maps very distant from the lactose operon on the *E. coli* chromosome and does not respond to any of the controls of the lactose operon, such as lactose repressor protein or induction by β galactosides.

The only other gene mapping in this region is a membrane determinant of tolerance to colicin E1 and so the *ebg-5* mutant identifies a new genetic locus. The mutation—or series of mutations—creating the lactose hydrolysing activity must presumably be located in an enzyme coded by this gene which is in wild type cells no relation of β galactosidase. One particularly intriguing question concerns the nature of the ancestral *ebg* enzyme in the deletion cells. What is its catalytic activity and how is this altered when it gains the ability to hydrolyse lactose? And, of course, it cannot but be interesting to isolate the enzymes coded by the successive *ebg* colonies and to determine their molecular changes. It is a long standing tenet of evolutionary theory that changes in protein activities lead a cell to gain the abilities appropriate for a new environment; but it is not often that such evolution can be followed through changes in one enzyme.

ELECTRON MICROSCOPY

Wide Applications

from a Correspondent

THE scanning electron microscope (SEM) is a relatively new addition to the range of instruments available to the physicist and biologist. Nevertheless it has established itself in a short time as a major research tool because of its adaptability, and also because of its inherent suitability for the time-resolved and dynamic *in situ* experimentation favoured for much current research. In this role, scanning microscopy is treated primarily as a means of obtaining quantitative data rather than as merely a source of interesting pictures. This theme was stressed by many speakers at a conference held at the University of Newcastle on July 3–5. Professor T. E. Everhart (University of California, Berkeley) demonstrated how the SEM could be used as a direct source of input data for a computer, and he also showed how this interaction between the computer and the microscope could lead to the direct production of

numerical information about a variety of effects in solid state physics. The use of the SEM as a means of obtaining crystallographic data was also described by many speakers.

A variety of methods are now being used for this purpose including the electron channelling technique (by Dr D. C. Joy, University of Oxford), the Kossel pattern technique (by Dr D. J. Dingley, University of Bristol) and a new approach using information contained in the angular distribution of the backscattered electrons (by Dr J. Venables, University of Sussex). Techniques for the study of the specimen chemistry, based on the analysis of the fluorescent X-ray spectrum stimulated by the electron beam, were reviewed by Dr J. V. P. Long (University of Cambridge) and a glimpse of the future was provided in the paper by Dr I. R. M. Wardell (Vacuum Generators Ltd, East Grinstead) describing the production of high resolution surface chemistry data by means of the Auger emission stimulated by the incident beam.

A major area of interest at this meeting was the use of the SEM in a scanning transmission mode of operation. The advantages of the SEM used in this way, compared to the conventional transmission electron microscope, were comprehensively reviewed for both physical and biological applications in papers by Professor L. Reimer (University of Munich) and Dr J. S. Wall (University of Chicago). Microscopes specifically designed for this application were described by several groups of workers including Dr A. N. Broers (IBM) who showed results from an instrument using a Latharum Hexaboride electron gun. Much attention was also focused on field emission guns as possible electron sources for this (and other) applications, and developments in this area were described by several workers including Dr R. J. Taylor (Cambridge Scientific Instruments Ltd) and Dr J. R. Banbury (AEI Ltd).

A full range of papers on straightforward applications of the SEM were also presented covering topics as diverse as studies of metal working procedures (by Dr S. Ramalingam, University of New York, Buffalo) and applications to forensic science (by Dr M. E. Taylor, West Midlands Forensic Science Laboratory). A very complete commercial exhibition allowed conference participants the chance to examine and compare the wide range of instruments and accessories now available. The conference was attended by more than 250 participants including many from Europe and the United States. Mr D. Kynaston (Cambridge Scientific Instruments Ltd) and Mr E. H. Boulton (University of Newcastle), the organizers, are to be congratulated for arranging a stimulating and timely meeting.

EARTH SCIENCES

San Andreas Watch

from a Correspondent

EARTHQUAKE prediction, present seismicity and strain accumulation, and past motions along the San Andreas fault as inferred from geological and geochemical evidence were the topics discussed at a meeting at Stanford University from June 20-23.

Great progress has been made by Dr J. C. Crowell (University of California, Santa Barbara) and others in unravelling fault motions in southern California. The San Gabriel fault is now believed to have been the main strand of the San Andreas system throughout the mid-Tertiary. It moved vigorously 10 to 20 million years ago and continued to slip up to 3 or 4 million years ago when the San Andreas fault became active. Early Miocene displacement along the San Gabriel may have amounted to 40-60 km, which would bring the total displacement for that period of 350 km or so into agreement with that observed in central California. North of the Transverse Ranges, however, offset of earlier strata suggests an additional 300 km of right lateral slip which has not yet been documented in southern California.

Dr C. Scholz (Lamont) made an interesting comparison between the Alpine fault in New Zealand and the great bend region of the San Andreas. In New Zealand the Warran fault has the most offset across it but, in response to changing regional stress patterns, the Hope fault is now the active branch. Most of the recent seismicity in southern California is associated with the San Jacinto fault, which supplanted the Banning-Mission Creek trace 3 or 4 million years ago. Uplift occurs in the central portion of the Alpine fault where it is cocked with respect to the regional slip vector; in California, a similar misalignment is manifested by thrust faulting in the Transverse Ranges. As the San Andreas is the most heavily instrumented fault in the world, it is encouraging to think that the knowledge gained from it may have applicability elsewhere.

On the subject of earthquake prediction, more questions were raised than answered. Such fundamentals as whether seismic activity should increase or decrease prior to a large event are unresolved. On the one hand, Drs W. Ellsworth and R. Wesson (NCER) have predicted a magnitude 4.5 event in the Bear Valley region based upon a seismic gap approach. Dr T. Hanks (Cal. Tech.), on the other hand, suggests that large earthquakes will occur in zones of above average seismicity, such as the southern half of the San Jacinto fault zone.

Dr R. Nason (USGS), noticing

accelerations in surface creep rates in the fault zone near San Juan Bautista, has predicted a magnitude 6 event on the 50 km section of the San Andreas immediately south of that town. But Dr R. Burford and Dr R. Lamson (NCER) have found that surface creep tends to follow seismic slip, occurring in response to deep motion rather than preceding it. Certainly, earthquakes cluster in areas where the rate of creep changes most rapidly with position along the fault or is abnormally low. The prediction by Dr A. Johnson (Stanford) of a magnitude 5.8 event near Hollister is based on this. Such behaviour indicates that creep is being retarded for one reason or other, possibly due to "sticky spots" on the fault plane at depth. The position of the creep meter with respect to these obstructions will determine the kind of creep behaviour recorded. Unfortunately, almost nothing is known about the location, extent or persistence of sticky spots, so that for any particular area whether accelerations or decelerations might signal an earthquake can only be determined empirically.

Then, inevitably, there were discussions of travel time ratios and variations on that theme. Reports of normal travel times prior to large events outnumbered reports of anomalous behaviour by four to two, but certain patterns emerge. Systematic t_s/t_p variations have only been observed in association with thrust faults and not necessarily even then. Since Whitcomb *et*

al. showed that most of the variation in t_s/t_p is due to changes in t_p , Dr C. Allen (Cal. Tech.) has looked for changes in compressional wave velocity prior to the San Fernando earthquake using records of mining explosions. His group failed to see any systematic change, contrary to Whitcomb's observations. Differences in seismic source and receiver station geometry with respect to the focal zone probably account for the disparate results. The ray paths of the pulses examined by Allen's group would have passed under the epicentral region at a depth of about 25 km. As virtually all of the seismic activity in California is confined to the upper 15 km of the Earth's crust, it is perhaps not surprising that no effect was observed. Whitcomb and coworkers also used remote stations, but because the sources were foreshocks, the ray paths through the focal zone were quite a bit shallower.

Dr I. Gupta (Mackay School of Mines, Reno) reported the use of variations in $t_{SH}-t_{SV}$ to predict the time of occurrence of earthquakes in central Nevada. This scheme is promising in that, if the gross features of the regional stress pattern are known, only one seismic station is required to monitor an area. Moreover, variations in $t_{SH}-t_{SV}$ are expected in regions of strike-slip and normal faulting.

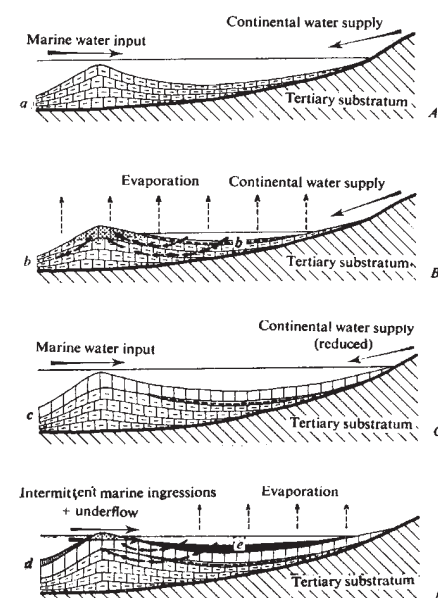
If earthquake prediction using seismic methods is in its infancy, two other methods in the foetal stage received attention. One, relying on the magneto-seismic effect, does not appear promis-

Past Climate of Tunisia

IN next Monday's *Nature Physical Science* a climatic history of southern Tunisia for the past 80,000 years is revealed from cores obtained from the Sebkh el Melah by Fontes and Perthuisot. The Sebkh el Melah is a coastal basin filled with recent post-Neotyrrenian deposits and the sediments reveal in order first a thin layer of saline mud with dolomite, magnesite and sometimes polyhalite. Below this layer is found a thin layer of mixed halite and gypsum with secondary polyhalite towards the inland. Underneath this is found a carbonate complex containing sands and clays with their upper parts rich in magnesite and/or huntite. Then there is a layer of clays and silts with gypsum.

The principal sedimentation events which occurred at Sebkh el Melah are shown in the diagram. The chief features of the climatic record which Fontes and Perthuisot deduce is that two high sea levels occurred at about 35,000 and 6,000 years ago. The first of these high seas is, of course, well known, and the existence of the second is often

discussed. The present work is another piece of evidence in favour of this second high sea level.



ing because anomalies on the order of one gamma will have to be extracted from a background noise of 40 or 50 gamma. The other, monitoring deep-seated resistivity changes, may prove to be the way of the future, as electrical resistivity is inherently more sensitive to changes in crack geometry and pore fluid contents than are seismic velocities.

There seem to be no serious objections to dilatancy in some form or other as being the cause of observed precursory phenomena. This is generally assumed to be coupled with condensed pore fluid flow, but Dr D. L. Anderson (Cal. Tech.) has suggested that the water-steam transition may be adequate. Water in voids at temperatures in excess of 100° C would vaporize if the void space increased sufficiently to drop the pressure. Vapour-filled interstices could be expected to behave much like dry ones; consequently compressional wave velocities would drop. As fluid moved in from surrounding areas and fluid pressure rose, the vapour would condense and the voids become water saturated again, raising the P-wave velocity. This rise in velocity will not necessarily happen before the earthquake. If the regional stress accumulates sufficiently quickly, voids may open as fast as fluid can move in and the fluid pressure will not rise. Thus, an earthquake may occur while the seismic velocity is at a depressed value.

In a seismically active area such as the San Andreas fault system, the crust may be dilatant all the time. It is important then that in either a water or a water-steam dilatancy model, the anomalous region is not the dilatant one, but the undersaturated one, the region of low pore pressure or dry voids. If this undersaturation fails to happen, as it would if stress accumulation were quite slow, then no seismic anomaly would precede an earthquake. Conversely, an undersaturated region exhibiting low seismic velocities could recover without experiencing an earthquake.

Given current understanding of the causes of shallow focus shocks and their likely precursors, the plea of Dr C. B. Raleigh (NCER) for restraint in predicting damaging earthquakes is altogether appropriate. Earth scientists must be wary of crying wolf. For one thing, it is not certain that the trends from limited observational evidence can be extrapolated to large events. For another, the social consequences of predicting a large earthquake should not be taken lightly. Earthquakes are not like hurricanes, for which the danger persists only over a short and well-defined period. Suppose the population was evacuated and the earthquake failed to occur within the predicted time interval?

LOW TEMPERATURE PHYSICS

Below One Millikelvin

from our Condensed Matter Correspondent

A TEMPERATURE below 1 mK has been reached by adiabatic demagnetization of magnetically diluted cerium magnesium nitrate. The experiment was performed at the Nuclear Physics Institute of the Czechoslovak Academy of Sciences at Rez, by M. Kolář, K. Svec, R. S. Safrata, J. Matas and T. Těthal (*J. low temp. Phys.*, **11**, 297; 1973).

The adiabatic demagnetization of paramagnetic crystals has been in use as a cooling technique since the 1930s and is, in essence, extremely simple. These crystals contain ions which possess net magnetic moments which are not aligned in any preferred direction but which are changing their orientations in a random way, due to the thermal vibrations of the lattice. By applying a magnetic field, these moments can all be aligned, which reduces the entropy of the system so that heat must be carried away to keep the temperature constant. If the crystal is then thermally isolated, so that its entropy cannot change, and the magnetic field slowly reduced to zero, it will cool because, in zero magnetic field, its entropy will correspond to a very much lower temperature. The ultimate limit on the final temperature which can be attained in this way is determined by the tendency of the magnetic ions to align spontaneously below a certain critical temperature T_c , due to their own mutual interaction. In order to minimize these interactions, and thus T_c , crystals are chosen which have very large unit cells so that the magnetic ions are well separated from each other. In pure cerium magnesium nitrate (CMN), for example, interactions between the magnetic cerium ions limit the final temperature to about 2 mK.

To reach even lower temperatures, the mutual interaction of the magnetic moments must be reduced, and one approach has been to turn to systems, such as copper nuclei, in which the moments are themselves smaller. In practice, although it is relatively easy to demagnetize copper nuclei adiabatically to about 10^{-6} K, the usefulness of the technique is limited by the great length of time (days or weeks) taken for the system of nuclear moments to transmit its "cold" to anything else. From this point of view, CMN is a more convenient refrigerant for cooling, for example, liquid ^3He , because equilibrium times are then typically of the order of minutes, although, of course, the final temperatures obtained using the pure salt are too high for some experiments.

An alternative way of reducing interactions between the magnetic ions, which was discussed in detail by B. M. Abraham, O. Brandt, Y. Eckstein, J. B.

Ketterson, M. Kuchnir and P. Roach of the Argonne National Laboratory (*Phys. Rev.*, **187**, 273; 1969), is to increase their average separation by replacing most of them by ions which, though chemically similar, are non-magnetic. These authors estimated that, with about 90% of the cerium ions in CMN replaced by lanthanum ions, it ought to be possible to reach a final temperature near 0.7 mK. This is the experiment which has now been performed by the group at Rez.

Temperature measurement near 1 mK represents a formidable problem and there is, as yet, no internationally agreed temperature scale in this region. It is customary to express experimental results in terms of a magnetic temperature T^* deduced from measurements of the magnetic susceptibility of CMN, which is assumed inversely proportional to the temperature (Curie's law). Using their diluted crystal, the Rez group report that they reached a final T^* of 0.86 mK, which they believe does not differ greatly from the real (thermodynamic) temperature, and which is in reasonable agreement with the theoretical predictions of the Argonne group.

The significance of this result must be seen in the context of the recent discovery of the new phases of liquid ^3He , which is known to undergo two separate phase transitions near 2 mK, only one of which can be reached by demagnetizing pure CMN. There is evidence to suggest that these new phases may have superfluid properties, and a period of intense experimental and theoretical endeavour is already in progress to try and understand their nature. They may be somewhat akin to the electron gas in a superconducting metal, but, alternatively, they may represent completely new states of matter.

Only one cooling technique, adiabatic compression of liquid ^3He across the solidification curve, has so far been successful in cooling the liquid through both transitions, and it suffers from the inevitable inconvenience that solid, as well as liquid, ^3He is always present in the cell once cooling has commenced. The Rez workers have now shown that research on both of the new phases should be greatly facilitated through use of magnetically diluted CMN as refrigerant.

Nature Subscriptions

THE *Nature* subscription list has been computerized. Each address label now carries a reference consisting of three letters and four numbers. Any queries relating to subscriptions should in future quote this reference.

Canadian Tropical Research in the West Indies

FINN SANDER

Bellairs Research Institute of McGill University, St James, Barbados, West Indies

Situated on the west coast of Barbados, the Bellairs Research Institute of McGill University is the only Canadian research and teaching laboratory in the tropics. It accepts, therefore, a special obligation to support Canadian research in that region, although it also has an interest in attracting scientists and students from around the world and maintains an open-door policy to all researchers with academic interests in the tropical environments.

INITIALLY, the research interests of the Bellairs Research Institute were directed primarily towards marine biology, but in the past decade they have broadened to include a spectrum of disciplines in science such as geography, geology and chemical and physical oceanography. During the same period the institute has expanded physically to satisfy a concomitant increase in demand for research and accommodation facilities. Also, additional analytical equipment, microscopic and photographic accessories, library holdings and boats have been acquired. The latter include a 54 foot research vessel, which is equipped with a winch, echo sounder and radio telephone and is capable of a wide range of hydrographic and biological collections.

More Popular

Expansion of the institute is only a byproduct of an increasing interest in the tropical environment of Barbados, the reason for which lies in the advantages the island presents to the visiting scientist. To the marine biologist, for example, the chief advantage is undoubtedly the diversity of communities encountered and their close proximity to the institute. Barbados offers research opportunities on coral reefs, rocky shores, sandy beaches, brackish water ponds and areas of shallow water flats of sand, mud or grass. The 100 fathom line lies only 1 mile offshore and deep oceanic water is present within a few miles of the coast.

Researchers have not hesitated to exploit this diversity. The ground work was laid by the first director of the institute, Professor J. B. Lewis, who described the coral reefs and coral communities¹, the fauna of the rocky shores² and the deep water benthos³. The thirty-six scleractinian species of coral found on the actively growing, fringing reefs along the western coast of Barbados (Fig. 1) are complemented by varied populations of sponges, anemones, molluscs, polychaetes, sea urchins and cucumbers and colourful reef fish. The coral reefs thus offer boundless scope for research and, indeed, members of the institute are presently conducting studies into the pattern of distribution of coral communities on the outer bank off the west coast. They hope to correlate the observed distribution with physical factors such as light, currents and movement of sediment and with biological interaction between the different species of corals and other benthic organisms.

The windward and leeward sides of the island experience marked differences in wave action and attendant exposure—a fact which renders studies on the zonation of the intertidal fauna of the rocky shores of Barbados extremely interesting, as wave action has been shown to be an important factor in extending the vertical range while exposure influences distribution.

Rich and diverse deep benthic communities of coelenterates, molluscs and echinoderms are readily accessible to the prospective investigator inasmuch as the island shelf is very narrow. Also, the close proximity of the island to offshore waters and its position in an area of relative ocean stability make it ideal for studies of dynamic production processes and for determining the "island mass" effect, a tendency to enhanced production in regions round oceanic islands. Recent investigations at the institute on these subjects have revealed lack of seasonal variations in the rate of primary production in this part of the tropical Western Atlantic Ocean⁴ and evidence suggesting that internal waves, which were shown to oscillate vertically in conjunction with high nutrient concentrations of deeper water, act as the principal causative mechanisms by which inshore island waters are enriched⁵.

There is also a varied marine flora around the coast of Barbados which includes extensive beds of the tropical marine angiosperm *Thalassia testudinum*. Rates of primary production by *Thalassia* rank amongst those of the most highly productive plants known⁶, and, until recently, the question of how high rates of production by *Thalassia* are maintained in notably nutrient-poor tropical waters was unanswered. The problem was resolved, however, when a graduate student working at the institute made the important

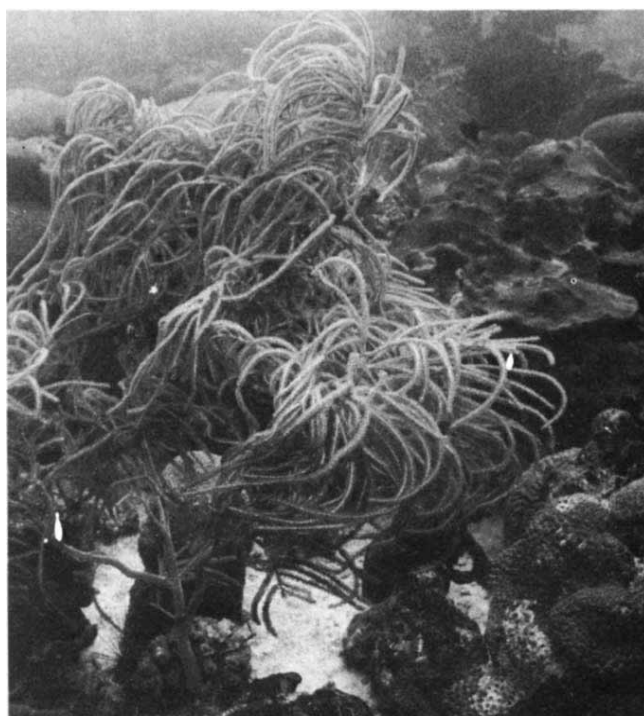


Fig. 1 A section of the prolific coral reef on the west (leeward) coast of Barbados.

discovery that nitrogen for growth of *Thalassia* is derived exclusively from gaseous nitrogen fixed by anaerobic bacteria in the rhizosphere⁷. It had previously been unsuspected that such a phenomenon might be important in the ecology of aquatic plants.

Oceanography and Geology

The island also holds certain advantages for the physical oceanographer. As an oceanic island it is in a favourable position for the study of detailed influences of a land mass on an important ocean current system. A large proportion of the water entering the Caribbean from the Atlantic flows into the channel between Barbados and Tobago. Barbados is thus in an excellent position for monitoring the direction and velocity of this flow. On the surface it would seem that this roughly east-to-west current system would tend to sweep away planktonic larvae of inshore invertebrates and reef fish. An important contribution towards understanding how the island's larval stock is maintained was made by another graduate student who suggested that a downstream system of vortices offered spawn and larval forms, released from the coastal current system, a chance of getting caught in the vortices and hence returned to inshore waters⁸.

Barbados also offers opportunities, some unique to the island, for research into problems of the geology of the Caribbean. Study of the deposition and diagenesis of carbonate sediment in the reef environment, the structure and facies of uplifted Pleistocene reefs of several ages, the effects of sea level changes in the Pleistocene and Holocene, and the influence of crustal plates on the historical geology of the Caribbean are problems open to attack through studies in Barbados.

The fringing reefs which grow from headlands along the west coast of the island, north of Bridgetown, supply carbonate sediment offshore⁹. A submarine ridge a few hundred metres offshore in a depth of about 15 m appears to be a drowned Pleistocene barrier reef^{10,11} along which scattered corals are now growing and submarine cementation is taking place¹². Although the fauna of the fringing reef is not as diverse as that of some other Caribbean islands, its proximity to the land and the institute makes it convenient for study of the growth of reef organisms¹³ and the binding, framebuilding and sediment-producing functions of the reef biota.

The elevated reefs and backcliffs which cover much of the island, excluding the Scotland district, have provided a natural laboratory for the examination of Quaternary reefs and the institute has played a significant part in this work. Deformed early Cainozoic, terrigenous and carbonate sediments form the highest parts of the island—the Scotland district on the north-east side. Pleistocene reefs, marking stages in the progressive rise of the island against a background of fluctuating sea levels, form concentric terraces around three sides of the Scotland district. Studies of the ages of these reef terraces have been made by a group from Brown University¹⁴ and on the north coast by James¹⁵. James has also studied the facies belts shown by these ancient reefs and the environment of the crusts forming on the limestone outcrops swept by salt spray on the north-eastern coast. He has shown that, contrary to one's natural expectations, the recrystallization and inversion of aragonite to calcite in coral skeletons during diagenesis take place from the axis of the corallite wall outwards towards its periphery.

Tectonics

Barbados occupies a key position for the understanding of the tectonics of the Caribbean and the relationship of the west Atlantic crustal plate, moving westwards, and the Caribbean plate, moving north-eastwards. Many problems

concerning the nature and history of the non-volcanic crustal rise associated with Barbados and its relationship to the volcanic arc of the Lesser Antilles remain to be resolved.

Geographers at McGill University have long recognized the research potential of the institute as a permanent facility with physical laboratory, library and draughting space as well as instrument storage and living quarters. Faculty and graduate research began more than a decade ago and has continued to the present, particularly in geomorphology and climatology and to a lesser extent in human geography. There are conspicuous climatic contrasts between the windward, eastern coast, and the leeward side of the island; these differences have been analysed in a continuing series of studies by Garnier and his colleagues, and have formed the basis of theoretical discussions of a model island in the trade wind belt.

Climatic and geological differences and variations between marine environments, wave incidence and energy on the east and west coasts are responsible for the striking contrast between the east coast, with high limestone cliffs and straight, predominantly silica-sand beaches unprotected by reefs, and the leeward shore with small beach cells of a low energy environment flanked by coral headlands. The institute facilities are being used to direct a series of long-term measurements, spread over a decade or more, of cliff and beach changes, including tracers and studies, cliff profiles and the dynamic changes of the cliff through stereophotographic and embedded steel pin observations (A. Richards and J. B. Bird, and P. P. Wong, unpublished). These studies have an immediate application in the preservation of the coral sand beaches on the west side of the island which form one of the most valuable natural resources for the tourist industry.

Subaerial geomorphic processes are being studied in the Scotland district where small watersheds have been instrumented for examination of run-off, sediment yield and slope change, all of which are spectacular in terms of morphological changes and land use in a tropical environment.

Although the Bellairs laboratory has proved most valuable for studies in the physical and biological areas of geography, the island's varying physical environments, and the historical evolution of society and the economy, have produced interesting contrasts between estate-dominated agriculture in the greater part of the island and peasant holdings in a few areas; these aspects are all studied at the institute.

Last but not least, field work is seldom interrupted on account of inclement weather. Barbados lies outside the principal hurricane zones and the climate is healthy and pleasant, tropical but tempered with trade winds. The annual temperature normally ranges between 75° F and 85° F, rarely falling below 68° F or rising above 88° F. More than 3,000 h of sunshine are recorded annually.

¹ Lewis, J. B., *Can. J. Zool.*, **38**, 1133 (1960).

² Lewis, J. B., *Can. J. Zool.*, **38**, 391 (1960).

³ Lewis, J. B., *Can. J. Zool.*, **43**, 1049 (1965).

⁴ Steven, D. M., *Mar. Biol.*, **10**, 261 (1971).

⁵ Sander, F., thesis, McGill Univ. (1971).

⁶ Odum, E. P., *Fundamentals of Ecology* (W. B. Saunders, Philadelphia, 1959).

⁷ Patriquin, D. G., thesis, McGill Univ. (1971).

⁸ Emery, A. R., thesis, McGill Univ. (1964).

⁹ Macintyre, I. G., *Mar. Geol.*, **9**, 5 (1970).

¹⁰ Macintyre, I. G., *Can. J. Earth Sci.*, **4**, 461 (1967).

¹¹ Macintyre, I. G., *Bull. Am. Ass. Petrol. Geol.*, **56**, 720 (1972).

¹² Macintyre, I. G., Mountjoy, E., and D'Anglejan, B. F., *J. sedim. Petrol.*, **38**, 660 (1968).

¹³ Stearn, C. W., and Riding, R., *Bull. Am. Ass. Petrol. Geol.*, **56**, 655 (1972).

¹⁴ Mesolella, K. H., Sealy, H. A., and Matthews, R. K., *Bull. Am. Ass. Petrol. Geol.*, **54**, 1899 (1970).

¹⁵ James, N. P., thesis, McGill Univ. (1972).

Information Transmission in a Diffusion-Coupled Oscillatory Chemical System

H. BUSSE & B. HESS

Max-Planck-Institut für Ernährungsphysiologie, 46 Dortmund, Rheinlanddamm 201, Federal Republic of Germany

An experiment on signal transmission in a chemical system involving the catalytic oxidation of malonic acid by bromate in an acidic medium is reported in this article.

Most chemical reactions proceed monotonically towards thermodynamic equilibrium, sometimes with the formation of intermediates which later disappear. But there are reactions in which intermediates and products appear periodically, such as the inorganic oscillating system described by Bray¹ and the oscillating advancement in a reaction system consisting of inorganic and organic compounds described by Belousov². Also, oscillatory variations of intermediate compounds have been detected in biochemical systems such as the glycolytic pathway or the oxidative processes in mitochondria³. All these reactions have been observed under conditions far from thermodynamic equilibrium.

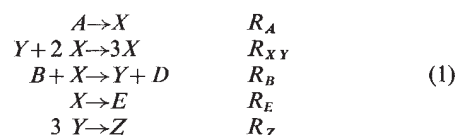
Synchronization of the phase of oscillations in different spatial locations is closely related to periodic time courses of chemical reactions. In suitable conditions, this leads to a spatial distribution of some components. Zhabotinsky⁴ has described a chemical system similar to the one of Belousov² which displays such phenomena. The experiments, performed in long tubes, revealed a spatial pattern moving along the tube. Later, such experiments were improved and extended^{5,6} demonstrating moving spiral, ring-shaped and more complex patterns. These patterns resemble structures observed during aggregation in a mutant of *Dictyostelium discoideum*⁷ and may be related to those encountered in nerve cells. These examples differ from non-biological ones in that the spatial distribution of the oscillatory system carries information; indeed, in the case of aggregation, it defines the direction of movement.

Because of the complexity of equation systems describing oscillatory and spatial effects we deal only with some simplified mechanisms. We know that quasi-stable singularities of any dimension may lead to a new class of states; thus, movement in a limit cycle results in an oscillatory reaction advancement and coupled with diffusion can generate spatial effects. Such effects occur in special conditions of stability or instability, far from thermodynamic equilibrium. The combination of oscillatory and spatial effects and a description of the mathematical treatment of a localized space-time dependent structure and space-time responses under specified initial conditions are described in refs 8 and 9.

Can diffusion-coupled oscillatory chemical systems be used to transmit information over a longer distance? Here we use the bromate-malonic acid system to demonstrate signal transmission. Because the mechanism of the system is not known in detail, we chose a chemical reaction scheme similar to that of ref. 10 as a model.

Elementary Chemical System

Lefever¹¹ described a simple chemical model system which has the essential properties of an oscillatory non-equilibrium state, that is, limit cycle behaviour. Analysis of the kinetic laws of this system demonstrated instability and periodicities in time and space as well as localized structures⁸. If one more trimolecular reaction (R_Z) is added, sequence (1) is obtained:



The corresponding network (Fig. 1) is derived from the chemical reaction scheme of ref. 12 (see also refs 13, 14). Neglecting all reverse reactions, setting all forward rate constants to unity and $A=1$, $B=5$ the system moves on a limit cycle. The time dependence of some variables in extreme non-equilibrium conditions is illustrated in Fig. 1.

The oscillatory behaviour of this system resembles that of a relaxation oscillator¹⁵. A concentration (charge) is accumulated

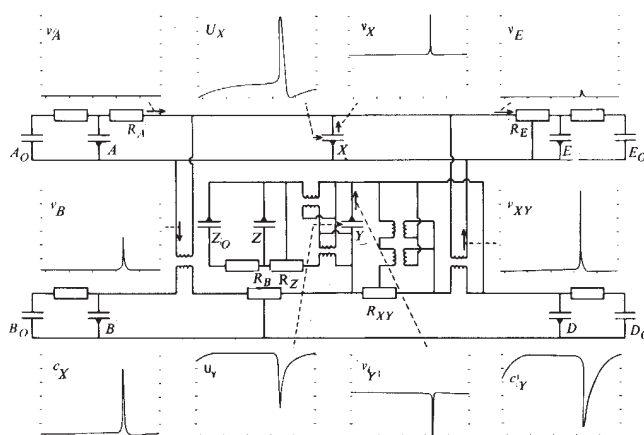


Fig. 1 Network of chemical reaction system (1): A , B , D , E , X , Y and Z are chemical compounds corresponding to energy storing capacitors. A_0 , B_0 , D_0 , E_0 and Z_0 are chemical compounds serving as reservoirs to maintain constant concentrations of A , B , D , E and Z . The oscillation proceeds by charging capacitor Y through R_B up to a threshold value against discharge through R_Z . Then reaction R_{XY} fires and discharges the capacitor Y . v is the rate of reaction. c_X and c_Y designate the variations in the concentration of X and Y . U_X and U_Y are the normalized chemical potentials. The fulltime scale is 52 units. The scaling unit starting from zero for v_A , v_B , v_E and v_{XY} is 15.6; the scaling unit for v_X and v_Y is about 19.2. Most of the times v_X and v_Y are close to zero. The scaling unit for c_X and c_Y is 1.53 starting from zero. The scaling unit for U_X and U_Y is 0.64.

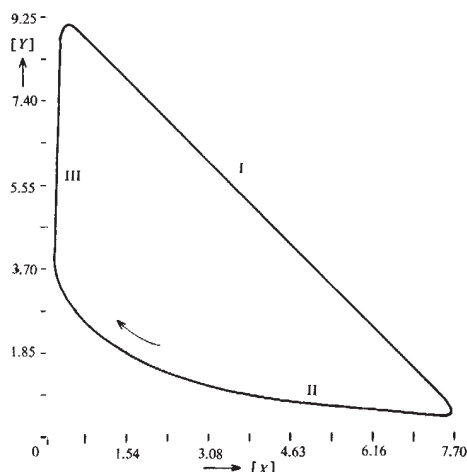


Fig. 2 Limit cycle of the chemical reaction scheme in Fig. 1. The conditions are the same as for Fig. 1. The scaling of concentration of X and Y is dimensionless. The motion on the limit cycle is discussed in detail by Lavenda *et al.*¹⁰ for a scheme comprising the reactions R_A through R_E . In part I the autocatalytic step R_{XY} determines the transformation of Y into X . In part II X disappears by the steps R_E and R_B while Y remains small. In part III, X remains small while Y is accumulated by the step R_B . The additional step R_Z contributes to the movement only at high concentrations of Y , at the end of part III. Here most of the Y produced by the step R_B is consumed by R_Z resulting in a very slow approach to the critical concentration, which activates the autocatalytic step of part I. This analysis clearly demonstrates that in different portions of the limit cycle different chemical processes determine the motion of the system.

up to its threshold value and subsequently the capacitor (Y in Fig. 1) is discharged and the process starts anew.

The triangular form of the limit cycle of the essential components (X and Y) (Fig. 2) indicates three different phases of the limit cycle.

To allow for diffusion processes the network must be extended by an additional resistive element describing the linear relation between the diffusion flux (I_D) and its concentration gradient:

$$I_D = D \Delta c / \Delta x = D c^* (e^{U_{out}} - e^{U_{in}}) / \Delta x \quad (2)$$

c^* , c are reference concentration and concentration respectively; x a space coordinate; D the diffusion constant, and U the normalized chemical potential¹².

The approximation for small concentration differences gives

$$I_D \approx D \bar{c} \Delta u / \Delta x \quad (3)$$

where $\bar{c}$ is the geometric mean of the concentrations at the ports and $\Delta u = U_{out} - U_{in}$. This can be used to approximate a continuum adequately by a lumped network of resistive elements representing diffusion processes.

Assuming that only X diffuses we obtain for a space element the two-port network shown in Fig. 3 (upper schematic).

A chain of such one dimensional two-ports (Fig. 3, lower schematic) represents a transmission line, composed of symmetrical T-type two-ports. Because any element of the two-port is passive the whole two-port is passive. In the limit of a great number of infinitesimal lumped elements the behaviour of the line approximates that of a one dimensional continuum¹⁴.

Analysis of Transmission Properties

To analyse properties of the transmission line its differential equation system obtained from rate processes of sequence (1) and the diffusion coupling between the line elements must be considered

$$\frac{dX_i}{dt} = A + X_i^2 \cdot Y_i - (B+1)X_i + q_x(X_{i-1} + X_{i+1} - 2X_i) \quad (4a)$$

$$\frac{dY_i}{dt} = B \cdot X_i - Y_i^3 - X_i^2 \cdot Y_i \quad (4b)$$

where $q_x = D_x / \Delta x$; D_x is the diffusion coefficient of X and Δx the length of the line represented by this element. i denotes the number of the oscillatory elements in the chain (Fig. 3, lower schematic). At $i=1$ and $i=n$ the concentrations $X_0 - X_1$ and $X_n - X_{n+1}$ are zero. The system of difference and differential equations⁴ is solved by Hamming's modified predictor-corrector method.

Without diffusion each element moves independently on a limit cycle. Because the outflow/inflow of compounds from the neighbouring element can retard/enhance the motion on the limit cycle, the diffusion helps to synchronize neighbouring elements. But this happens only if the system is sensitive to an addition or subtraction of the compound.

There may be parts of the limit cycle where the motion is nearly independent of the state of the neighbouring elements and other parts where the motion is strongly dependent on them. Further, during the sensitive part the diffusion leads to an equilibration of the phase differences between neighbouring cycles.

In system (1) the sensitive part of the limit cycle is exemplified at the end of portion III (Fig. 2). If, in this portion of the limit cycle, compound X is added to an oscillatory element the autocatalytic process (portion I) starts considerably earlier than otherwise. All elements which show a high Y and a low X concentration will immediately start their autocatalytic process when the neighbouring element has a sufficiently higher concentration in X ; as soon as an element moves into the sensitive part of its limit cycle the interaction with its neighbour determines its future motion. In a chain of oscillatory elements all having high Y and low X concentration, the element which first starts its autocatalytic process will force its neighbour to follow. If the autocatalytic process of the first element is triggered externally, a triggered pulse moves along the chain.

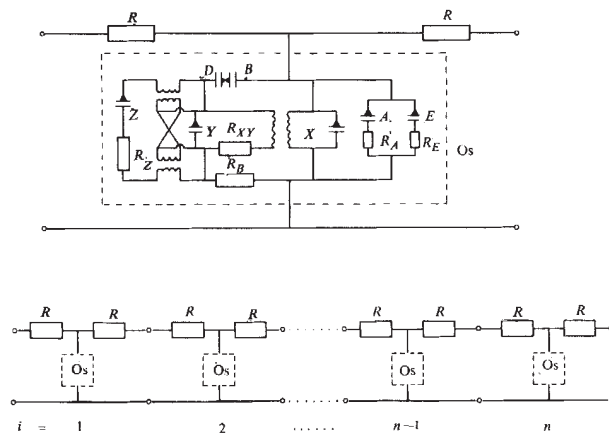


Fig. 3 The upper network is a symmetrical T-type four terminal network comprising the two resistors R originating from the diffusion process and the cross linking network enclosed in the dashed box, O_s . The O_s network is a rearranged drawing of the network given in Fig. 1 and the diffusive resistors R are added. Finally, all connexions not carrying a net current are omitted and the pools are eliminated. In the lower network a series of these two-ports is arranged in a chain in order to form a transmission line, composed of equal oscillatory units.

To achieve an optimized transmission the concentration of Y of system (1) should be maintained for a long time in the sensitive phase below its critical value. Then the pulse might travel a long distance before it reaches the limit where it interferes with the natural frequency of the oscillatory elements (step R_Z of system (1)).

A computation of a transmission line is represented in Fig. 4. Three trigger pulses at different time intervals are given to the first oscillatory element in the line. The signals run along the line almost undistorted and with nearly constant velocity.

Experimental Signal Propagation

Under suitable conditions the catalytic oxidation of malonic acid by bromate shows a wave-like distribution of the catalyst in space and time⁴. Also, the oscillatory oxidation can be influenced by ultraviolet flashes¹⁶. These phenomena may be combined to demonstrate the initialization of a propagating wave by ultraviolet radiation.

A typical experiment is carried out in a Petri dish onto which an ultraviolet beam is directed. The beam of a 450 W xenon-arc filtered for ultraviolet is focused to a diameter of about 0.4 cm on the solution layer, approximately 2 mm thick. After the solution is phase balanced by shaking, the "light" pulse is switched on in the blue phase of the oscillation for 2 to 30 s.

A wave propagates from the centre of the initiation with nearly constant velocity until after about 1 to 2 cm the wave disappears in the oscillation of the bulk solution (Fig. 5). During the next period, at the spot illuminated before, the solution turns blue before the bulk solution turns blue and again a wave starts from this position, disappearing in the oscillation of the bulk solution (see Fig. 5d).

Significance of the Experiment

Because no adequate chemical reaction scheme for the malonic acid-bromate or other oscillatory chemical systems is available, a comparison of experimental and theoretical results can only be qualitative.

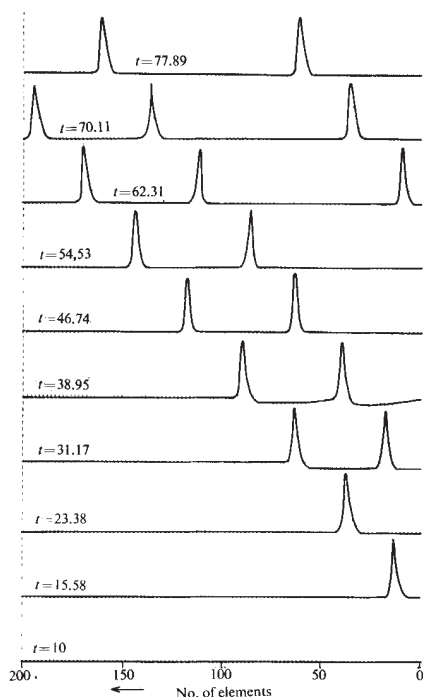


Fig. 4 Computer output of a transmission line demonstrating the propagation of pulses by which information is transferred. For 200 elements of the line the concentration of X is plotted at time t . The number of elements is plotted right to left. The curves are generated by sequence (1). The data for the oscillatory circuit are those of Fig. 1. The rate constant for step R_z is chosen 8.992×10^{-4} and the coupling constant of the diffusion $q_x = 0.35$. Here, the first element is triggered by a series of three pulses with distinct time intervals.

The common qualitative property in our theoretical system and the experimental system is the motion on a quasi-stable limit cycle. This cycle must have at least one portion that can be perturbed by a superimposed diffusional flow of chemical substances. Although a linear one-dimensional motion is computed, whereas the experiments show moving rings, the common feature of the initiation of a propagating wave remains.

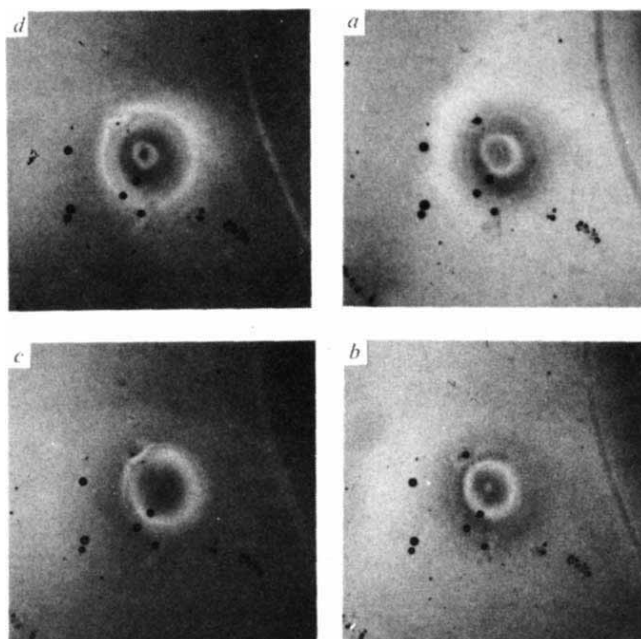


Fig. 5 Propagation of a signal in an oscillatory chemical reaction system. The initial concentrations of the solutions are

$[\text{KBrO}_3] = 0.35 \text{ M}$; $[\text{H}_2\text{C}(\text{COOH})_2] = 1.2 \text{ M}$;
 $[\text{Ce}(\text{SO}_4)_2] = 3.9 \times 10^{-3} \text{ M}$; $[\text{Ferrioin}] = 0.48 \times 10^{-3} \text{ M}$ in $3 \text{ N H}_2\text{SO}_4$

About 15 min after initiation of the reaction by addition of the catalyst the ultraviolet pulse is given and shortly afterwards pictures are taken with an interval of 0.5 s. The sequence of the pictures shown is clockwise from (a) to (d). The white ring represents the blue outward moving wave, the spot in the centre was irradiated. Further explanations see text. In d, the following wave is already forming. Other conditions do not show both wave rings simultaneously, but the irradiated spot always turns blue ahead of the bulk solution to form a new wave centre.

Other types of triggering in the solution can be used as information input, because in the solution the initiation should be independent of the propagation.

A combination of two or more signals in a specified way may be considered as an operation on the information contained in the signals; such combinations may lead to a network made of "chemical wires".

We thank Mr R. Schüller for programming advice and Mr H. Schlüter and Miss M. Böhm for technical assistance.

Received December 8, 1972; revised March 6, 1973.

- Bray, W. C., *J. Am. Chem. Soc.*, **43**, 1262 (1921).
- Belousov, B. P., *Sb. Ref. Radiats. Med. za 1958* (Medgiz, Moscow, 1959).
- Hess, B., and Boiteux, A., *Ann. Rev. Biochem.*, **40**, 237 (1971).
- Zhabotinsky, A. M., *Symp. Oscill. Processes in Biol. Chem.*, 252 (Academic, New York, 1967).
- Zaikin, A. N., and Zhabotinsky, A. M., *Russ. J. phys. Chem.*, **45**, 147 (1971).
- Winfree, A. T., *Science, N.Y.*, **175**, 634 (1972).
- Gerisch, G., *Naturwissenschaft*, **58**, 439 (1971).
- Herschkowitz-Kaufman, M., and Platten, J. K., *Bul. Classe Sci.*, **57**, 26 (1971).
- Romanovskii, U. M., and Sidorova, G. A., *Proc. Symp. Oscill. Processes in Biol. Chem.*, 258 (Academic, New York, 1967).
- Lavenda, B., Nicolis, G., and Herschkowitz-Kaufman, M., *J. theor. Biol.*, **32**, 283 (1971).
- Lefever, R., *J. chem. Phys.*, **49**, 4977 (1968).
- Hess, B., Chance, E. M., Busse, H., and Wurster, B., *Proc. Eighth FEBS-Meeting*, **25**, 119 (Amsterdam, 1972).
- Oster, G. F., Perelson, A. S., and Katchalsky, A., *Nature*, **234**, 393 (1971).
- Oster, G. F., Perelson, A. S., and Katchalsky, A., *Q. Rev. Biophys.* (in the press).
- Kohlrausch, F., *Praktische Physik*, **2**, 151 (Stuttgart, 1962).
- Vavilin, V. A., Zhabotinsky, A. M., and Zaikin, A. N., *Russ. J. phys. Chem.*, **42**, 1649 (1968).

Immunological Relationship of DNA Polymerase from Human Acute Leukaemia Cells and Primate and Mouse Leukaemia Virus Reverse Transcriptase

GEORGE J. TODARO & ROBERT C. GALLO

Viral Leukemia and Lymphoma Branch and Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20014

A reverse transcriptase isolated and purified from a case of human acute myeloblastic leukaemia cells has both biochemical and immunological properties similar to that of known type C leukaemia virus reverse transcriptases and, in particular, to the enzyme from primate type C virus.

ONE approach to determining if information derived from type C RNA tumour viruses is present in human leukaemic cells is to attempt to find viral proteins in ostensibly virus-free cells. When DNA polymerases are isolated from cells infected with virus, two principal cellular DNA polymerases can be separated and distinguished from the viral reverse transcriptase both on biochemical and immunological criteria¹⁻⁴. An enzyme with many of the biochemical properties of the viral reverse transcriptase has been found and partially purified from some human leukaemic cells⁵⁻⁷. The human leukaemic enzyme is isolated in a cytoplasmic particulate fraction, and, like viral reverse transcriptase, it catalyses an endogenous, ribonuclease (RNase)-sensitive DNA synthesis⁵⁻⁸. Various biochemical properties have made it possible to distinguish the viral DNA polymerase from the principal normal mammalian cell DNA polymerases^{1-4,9}. There are, however, several viruses that are not members of the leukaemia-sarcoma (type C) virus group, but nevertheless contain reverse transcriptase activity and 70S RNA. These include the mouse mammary tumour virus (a type B virus); visna virus and its relatives; the various "foamy" viruses; and a virus isolated from a monkey breast tumour, the Mason-Pfizer monkey virus^{3,10,11}.

Gerwin *et al.* initially demonstrated that the polymerase activity of leukaemia virus particles can be inhibited by antibody to the viral enzyme¹². They found that serum from rats carrying a transplantable sarcoma producing a mouse sarcoma virus contained an antibody to the mouse type C virus polymerase. Subsequent studies demonstrated that antibody to partially purified viral enzymes could be prepared^{13,14}, and this antibody would specifically inhibit the catalytic activity of the viral enzyme without inhibiting either of the two major cellular DNA polymerases¹. Antibody to the mouse leukaemia virus enzyme was found to inhibit other rodent type C virus enzymes (hamster, rat) and also the cat leukaemia virus enzyme. Antibody to the polymerase of avian type C viruses would only inhibit the enzyme from other avian type C viruses, but would not inhibit the polymerases of the mammalian viruses containing reverse transcriptase^{14,15}.

The recent demonstration that type C viruses could be directly isolated from certain primates¹⁶⁻¹⁸ led to the observation that antibody prepared to the purified polymerase of these viruses would inhibit the primate type C viruses, but would only minimally affect the type C virus reverse transcriptase of lower

mammals¹⁹. Thus a more precise determination of the relationship of this human leukaemic reverse transcriptase to known viral reverse transcriptases might be obtained using an immunological approach.

These observations prompted this study in which we report on the immunological properties of the human leukaemic reverse transcriptase tested with antisera previously prepared to reverse transcriptase of various RNA tumour viruses. Little or no relation to type B and avian type C virus was found. But we find that an enzyme purified from a human acute myelogenous leukaemia patient is closely related to mammalian type C virus reverse transcriptase, especially to reverse transcriptase from primate type C virus.

Source of Human Leukaemic Cells

Patient HL3, the source of cells used for isolation of the enzyme for most of the studies reported here, is a 37-yr-old man who presented with a recent history of bruising easily, bleeding and tender gums. There was no family history of either neoplasia or hereditary disorders. The pertinent findings on physical examination were marked gingival hypertrophy, lymphadenopathy, petechiae of the lower extremities, and hepatomegaly. His bone marrow was extremely hypercellular, most cells being atypical blast cells, most of which contained Auer rods. A diagnosis of acute myeloblastic leukaemia was made. His leukocyte count was elevated to 84,000 cells mm⁻³. An interesting finding was the presence of a pseudodiploid clone with a Philadelphia-like chromosome categorized in the G group. This chromosome has remained in spite of the fact that the patient was put into complete clinical and haematological remission (K. McCredie, personal communication). After cells were obtained for enzyme studies, this patient was treated with combined chemotherapy and has been in remission since.

Isolation of Leukaemic Cell Polymerase

Approximately 4×10^{10} blast cells (40 g) were obtained from the peripheral blood of the untreated patient by leukaphoresis. The cells were separated from other blood components as described previously²⁰. After removal of nuclei, a particulate fraction was obtained from a high speed post-mitochondrial cytoplasmic fraction. The "pellet" was suspended in buffer and an aliquot was applied to a sucrose gradient for equilibrium density gradient centrifugation to estimate the buoyant density of the endogenous DNA polymerase activity (Fig. 1). Simultaneously, the buoyant density of Rauscher leukaemia virus (RLV) was determined by assaying its DNA polymerase activity in an identical gradient. As shown in Fig. 1, both banded at peak density of 1.16 g ml⁻¹. The remaining leukaemic "pellet" suspension was applied to a glycerol gradient to begin further purification. After centrifugation for 100,000g for 1 h, the fractions containing endogenous and RNase-sensitive DNA polymerase activity were combined, dialysed, and concentrated. The enzyme was then extracted with KCl and 'Triton' X-100 and chromatographed in turn on DEAE cellulose, phosphocellulose, and

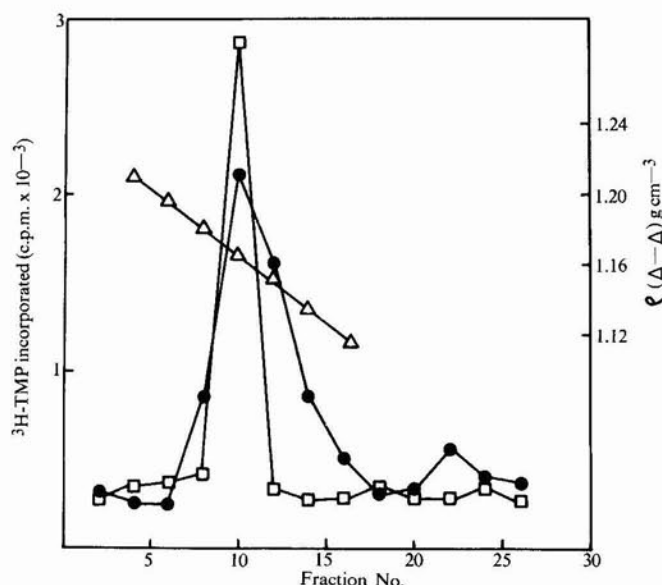


Fig. 1 Equilibrium sucrose density gradient centrifugation of human leukaemic pellet and Rauscher leukaemia virus (RLV). An aliquot (1 ml) of the sample was layered on 10 ml of 20–50% (w/w) linear sucrose gradient prepared in 0.01 M Tris-HCl (pH 7.5), 0.1 M NaCl, and 1 mM EDTA. The samples were centrifuged for 22 h at 60,000g in a 'Spinco' SW41 rotor. After centrifugation, 0.2 ml fractions were collected and the densities of every other fraction determined by refractive index measurement. Aliquots of 10 μ l from every other fraction were assayed for endogenous DNA polymerase activity in a 0.05 ml standard reaction mixture. The reaction mixture contained 50 mM Tris-HCl (pH 7.5); 60 mM NaCl; 5 mM DTT; 0.05% 'Triton' X-100; 80 μ M each of dATP, dCTP, dGTP; and 5.6 μ M 3 H-dTTP (8,100 c.p.m. pmol $^{-1}$). Five mM magnesium chloride was used for RLV. The samples were incubated at 37° C for 1 h. $\square$, RLV; $\bullet$, leukaemic 60 K pellet.

'Sephadex' G200 column chromatography⁵. This enzyme is enzyme (2) described in ref. 5, except that it was then treated with 1% 'Triton' X-100; 0.028 β -mercaptoethanol; 0.6 M KCl; and 0.05 M Tris (pH 7.0); and then chromatographed on a 'Sephadex' G100 column.

Template Properties for Polymerases

Table 1 shows some of the properties of the enzyme preparations used in the present studies with Mg²⁺ as the divalent cation. The viral polymerase shows a decided preference for poly (rA)-(dT)₁₂₋₁₈ as compared with poly (dA)-(dT)₁₂₋₁₈ as template. The ratios for the feline and the woolly type C polymerases were 12:1 and 6:1, respectively, very different from the values with the two principal DNA polymerases from normal (PHA stimulated) lymphocytes. The leukaemic cell polymerases showed a preference for poly (rA)-(dT)₁₂₋₁₈ (ratio 5:1). Similarly, the principal normal lymphocyte DNA polymerases show a much greater preference for activated salmon sperm DNA than poly (rA)-(dT) in contrast to the viral and leukaemic cell polymerases. The leukaemic cell enzyme, like the viral enzymes, also shows an endogenous ribonuclease sensitive reaction and was able to make a DNA product using 70S RNA from avian myeloblastosis virus and transcribes poly (rC) in the presence of (dG)₁₂. Thus, the biochemical properties of the human leukaemic polymerase are very different from the two principal cellular DNA polymerases.

Immunological Properties

Various viral reverse transcriptases were isolated and antisera were prepared in New Zealand white rabbits as previously described^{14,19}. Control sera used in each study were obtained from the pre-immunized rabbits. DEAE cellulose chromatography purified IgG from control sera and from the immune

sera were used in all the antibody inhibition studies. Antibody to the AMV polymerase, prepared in rats, was donated by Dr Robert Nowinski.

With the gibbon virus antipolymerase IgG, the human leukaemic cell enzyme, HL3 is inhibited almost as efficiently as the enzyme to which antibody had been prepared (Fig. 2).

The two known primate type C virus polymerases and the human leukaemic cell polymerase were comparably inhibited, but reverse transcriptases from two primate viruses that are not type C viruses (Mason-Pfizer monkey virus and simian foamy virus type 3) and from two non-primate type C viruses (feline leukaemia virus and Rous sarcoma virus) were not inhibited.

The gibbon antipolymerase IgG inhibited HL3 very efficiently (80% with 30 μ g IgG) but was less effective with similar DNA polymerase isolated from a patient with acute lymphoblastic leukaemia (patient HL5) (only 40% inhibition with 60 μ g IgG) (Fig. 3). Most human leukaemic cells that have been studied, unlike HL3, have not shown high levels of reverse transcriptase activity, and their polymerases are not inhibited (less than 40% with 60 μ g IgG) by the gibbon virus antipolymerase IgG. Both the control rabbit IgG and antiserum to the feline leukaemia virus enzyme failed to produce any detectable levels of inhibition over the range tested.

Using a potent antiserum to type C virus polymerase from a species more distantly related to man (the mouse), it is seen (Fig. 4) that less than 1 μ g of IgG gives detectable inhibition in the homologous system (anti-mouse virus (pol) IgG vs mouse virus polymerase). But at roughly ten times higher antibody concentrations this IgG is able to inhibit the gibbon ape virus polymerase and the human leukaemic cell polymerase, HL3. The curve for inhibition of polymerase activity with increasing levels of antimouse (pol) IgG was essentially the same for the gibbon virus enzyme and the leukaemic cell enzyme. By contrast, another primate virus, but not a type C virus, the

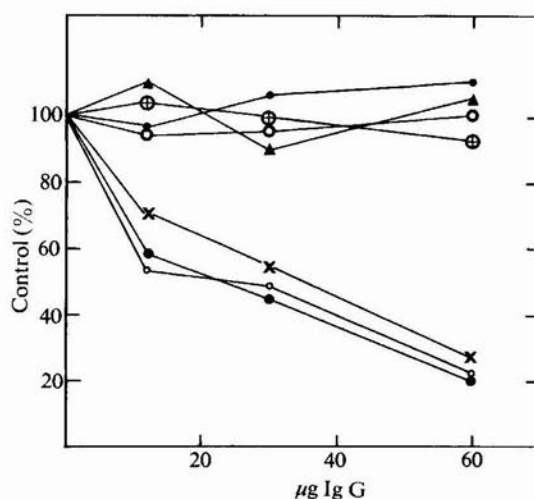


Fig. 2 Inhibitory effect of antibody (IgG) to gibbon ape virus reverse transcriptase on various reverse transcriptases. Each reaction mixture contained in 0.060 ml: 0.05 M Tris-HCl (pH 7.8); 0.06 M potassium chloride; 0.005 M manganese acetate; 0.002 M dithiothreitol; 0.05% 'Triton' X-100; 0.02 A₂₆₀ poly (rA)-oligo (dT)₁₂; and 2×10^{-5} M 3 H-TTP (50,000 c.p.m. pmol $^{-1}$) and was incubated at 37° C for 60 min. Incorporation in the absence of added IgG with each enzyme preparation was between 5,000 and 12,000 c.p.m. All reactions were linear with time and were assayed at enzyme levels where incorporation was proportional to enzyme. Each viral enzyme was purified by 'Sephadex' G100 chromatography before use. The source of each has been described before. The human leukaemia cell enzyme was purified as described in the text and in more detail elsewhere^{5,6}. Those reverse transcriptases showing inhibition are: $\circ$, gibbon; $\times$, woolly monkey; $\bullet$, HL3. Those not inhibited are: $\oplus$, Mason-Pfizer monkey; $\circ$, simian foamy virus type 3; $\bullet$, feline leukaemia virus; Δ , Rous sarcoma virus.

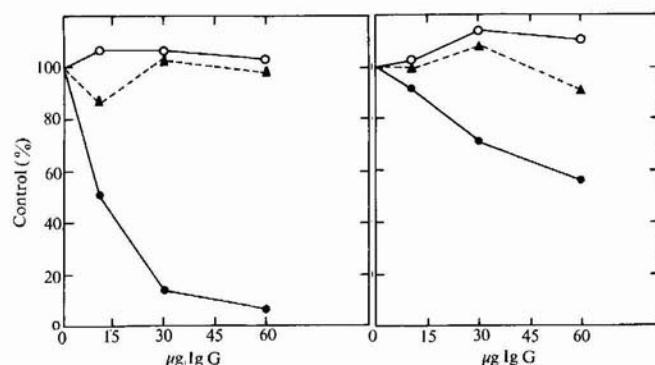


Fig. 3 Inhibition of leukaemic cell enzymes by control, antipol (FeLV), and antipol (gibbon). Reactions are as indicated in the legend to Fig. 2. Left: human acute myelogenous leukaemic polymerase, HL3. Right: human acute lymphocytic leukaemic polymerase, HL5. An enzyme partially purified from HeLa cells that has some properties analogous to viral reverse transcriptase²⁴ was provided by Dr A. Weissbach. In experiments not shown, it was partially inhibited (30–50% with 60 µg IgG) by antipol (gibbon), like HL5.

Mason-Pfizer monkey virus (M-PMV), was not inhibited, nor was DNA polymerase peak 1 from either normal human lymphocytes⁹ or normal human brain³ inhibited by up to 60 µg of IgG. The two principal DNA polymerases from normal human tissue (brain and PHA-stimulated blood lymphocytes) have not been inhibited by any of the antiviral polymerase sera, as previously reported for the two principal mouse DNA polymerases¹.

Another potent antiserum, prepared to the avian myeloblastosis virus enzyme¹⁵, was also found to produce substantial inhibition with less than 1 µg IgG of enzyme activity in the homologous system (that is, tested against the AMV enzyme). In this case, however, none of the mammalian viral enzymes were inhibited, even with 50 µg IgG (Fig. 5). HL3 and HL5 were, similarly, not inhibited. The antimouse virus (pol) IgG is able to inhibit the primate virus and human leukaemic cell polymerase, but the antiavian virus (pol) IgG is not.

The human leukaemic enzyme is also significantly inhibited by antibody to reverse transcriptase from the only other type C primate virus presently available, the simian sarcoma virus obtained from a woolly monkey¹⁶. In Table 2 we compare the relative inhibitory activity of antibodies prepared against the various viral reverse transcriptases on the human leukaemic enzyme. The HL3 enzyme is most closely related to the primate type C viruses, especially the gibbon ape type C virus.

Immunological Cross Reactions

The cells used in these studies were not tissue culture cells but fresh blood leukocytes, so that laboratory contamination is not a concern. We conclude, then, that at least some human leukaemic cells contain a polymerase with both biochemical and antigenic properties of reverse transcriptase of type C virus, but especially closely related to the polymerase of primate type C virus. It will be of interest now to determine the generality of this observation and if there is some correlation of the

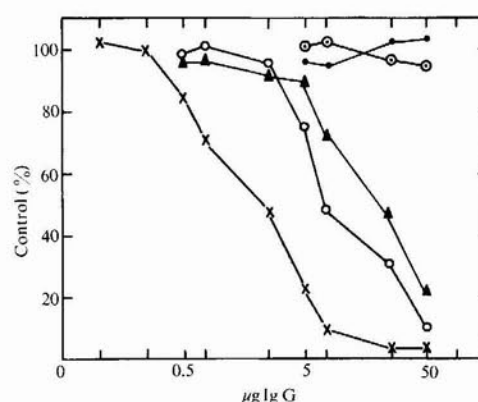


Fig. 4 Inhibitory effect of antibody to mouse leukaemia virus reverse transcriptase on various reverse transcriptases. Reactions are as in the legend to Fig. 2. x, mouse leukaemia virus polymerase; ▲, gibbon ape virus polymerase; ○, human leukaemic polymerase, HL3; ●, Mason-Pfizer monkey virus polymerase; ○, normal human cell polymerase (peak 1).

Table 1 Summary of Template Properties of DNA Polymerases of Viral and Cellular Origin

Source of DNA polymerase	Endogenous reaction*	Ratio†		Response to‡	
		Poly (rA)-(dT) ₁₂₋₁₈ poly (dA)-(dT) ₁₀	Poly (rA)-(dT) ₁₂₋₁₈ Activated DNA	poly (rC)-(dG) ₁₂ (pmol/10 µg protein)	AMV 70S RNA (pmol/10 µg protein)
Human leukaemic leukocytes					
HL3	+	5:1	7:1	4.0	5.1
Type C viruses					
FeLV	+	12:1	1:1	47	19.8
Woolly monkey	+	6:1	1:2	46	17.3
Human normal leukocytes					
Peak 1	—	1:12	<1:100	<0.05	0.01
Peak 2	—	<1:100	<1:100	<0.05	0.01

All DNA polymerase assays were carried out at 37° C for 1 h in standard 0.5 ml reaction mixtures containing 50 mM Tris-HCl (pH 8.3); 5 mM magnesium chloride; 30 mM sodium chloride; 5 mM dithiothreitol; and 10 µM ³H-TTP (8,100 c.p.m. pmol⁻¹). In the assays with "natural" templates, that is, the endogenous reaction, activated DNA, and AMV 70S RNA, the reaction mixtures also included 80 µmol each of dATP, dCTP, and dGTP. Assays with the synthetic templates poly (rA)-(dT)₁₂₋₁₈ and poly (dA)-(dT)₁₀ contained only 80 µmol dATP and the labelled nucleotide; assays with poly (rC)-(dG)₁₂ contained 10 µmol ³H-dGTP (6,930 c.p.m. pmol⁻¹). The exogenous template concentrations were 50 µg ml⁻¹. The two principal DNA polymerases from human normal leukocytes were prepared as previously described⁹. Poly (rA)-(dT)₁₂₋₁₈ was obtained from Collaborative Research, Waltham, Massachusetts. Poly (dA)-(dT)₁₀ was obtained and prepared as previously described⁵. AMV was obtained from Dr Joseph Beard. The 70S RNA was isolated from this virus as described elsewhere²⁸.

* This is the endogenous ribonuclease-sensitive reaction of the cytoplasmic pellet fraction. The template-primer is nucleic acid in the pellet. This activity bands in 1.16 µg ml⁻¹ density regions of a sucrose gradient as shown in Fig. 1. The ribonuclease is pancreatic RNase (20 µg ml⁻¹ with 0.2 M NaCl).

† All assays were tested with magnesium as the divalent cation. The enzymes are free of terminal transferase activity; assays with template alone, poly (rA) or poly (rC), or with the primer alone, (dT)₁₀ or (dG)₁₂, showed no detectable activity with magnesium or manganese. Activated DNA is deoxyribonuclease treated native salmon sperm DNA.

‡ (dT)₁₀, 10 µg ml⁻¹, is added with AMV 70S RNA. In the absence of (dT)₁₀, synthesis of DNA from viral RNA catalysed by the leukaemic enzymes has varied between 0.5 and 3 pmol/10 µg of protein. With the human leukaemic polymerases, it was shown that heteropolymeric portions of the viral 70S RNA were transcribed, as labelled dCTP, dGTP, and dATP were also incorporated into the newly synthesized DNA^{5,6}. In addition, the DNA products hybridized back specifically to the template RNA (AMV 70S RNA) and not to heterologous RNA (for example, MS2 RNA). For the synthesis of DNA from AMV RNA in the absence of added (dT)₁₀ the product hybridizes back to AMV RNA and not to MS2 RNA, MuLV RNA nor to poly (rA).

presence of this enzyme with various clinical states of leukaemia (onset, remission, relapse and so on). It will also be of great interest to perform the reciprocal immunological experiments in which antibodies prepared against the human leukaemic reverse transcriptase are tested against the known viral enzymes.

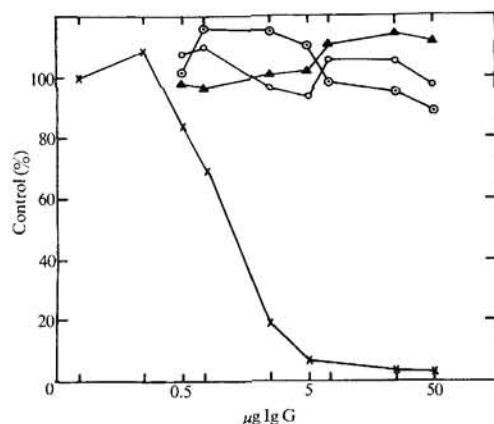


Fig. 5 Inhibitory effect of antibody to avian type C virus reverse transcriptase to various reverse transcriptases. The preparation of the antibody is described elsewhere¹⁵. Reactions are as in legend to Fig. 2. x, AMV polymerase; ▲, gibbon ape virus polymerase; ○, human leukaemia, HL3; ○, Mason-Pfizer monkey virus polymerase.

Various studies in mouse cells have now revealed partial expression of type C viral genetic information in supposedly virus-free cells, both by nucleic acid hybridization and by radioimmunoassay for the type C virus group specific (gs) antigen^{21,22}. The results described here do not, therefore, indicate that a whole virus particle is being produced by leukaemic cells. The possibility still exists that the HL3 polymerase is produced by the cell and shares immunological determinants and biochemical properties with the type C viral polymerase. In this respect, a reverse transcriptase has recently been described in normal, presumably virus-free, chicken cells. Immunological studies so far indicate, however, that this is not related to the reverse transcriptase of the known avian type C viruses²³. The enzyme found in HL3 can be distinguished from a third DNA polymerase activity in human cells described by Fridlender *et al.*²⁴ and McCaffrey *et al.*²⁵ because it is able to use the template poly (rC)-(dG)₁₂ and can transcribe the heteropolymeric portions of 70S viral RNA. The activity isolated from other human leukaemic cells, such as HL5, may well be predominantly, or entirely, the enzyme of Fridlender *et al.*²⁴; both are partially inhibited by the gibbon type C virus antipolymerase. Patient HL3 seems to be

unusual in the extent of the expression of an enzyme activity identical to or closely related to the viral reverse transcriptase.

If the human leukaemic reverse transcriptase is coded for by viral genes, the present studies do not distinguish between the expression of endogenous type C viral information²⁶ in the human leukaemic cells or exogenous infection by a virus from the outside²⁷. The finding of this enzyme in human leukaemic cells, then, does not establish the mechanism of cancer causation, but provides evidence that human tumour cells have at least one expression of the type C virus, the reverse transcriptase. If subsequent experiments can confirm the general existence of a type C virus-specific marker in human cells, it will be important to determine whether this activity is only found in neoplastic cells or if it is generally present in proliferating human cells or in immature cells.

We thank Drs J. Freireich, K. McCredie, E. Henderson and B. Leventhal for providing the clinical materials. We also thank Drs W. Parks, E. Scolnick and R. Nowinski for providing antisera, and Drs P. Sarin, D. Livingston and B. Talbot for suggestions. This work was supported in part by contracts from the Special Virus Cancer Program (National Cancer Institute).

Received April 24, 1973.

- ¹ Ross, J., Scolnick, E. M., Todaro, G. J., and Aaronson, S. A., *Nature new Biol.*, **231**, 163 (1971).
- ² Goodman, N. C., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2203 (1971).
- ³ Scolnick, E. M., Parks, W. P., Todaro, G. J., and Aaronson, S. A., *Nature new Biol.*, **235**, 35 (1972).
- ⁴ Robert, M. S., Smith, R. G., Gallo, R. C., Sarin, P. S., and Abrell, J. W., *Science*, **N.Y.**, **176**, 798 (1972).
- ⁵ Sarngadharan, M. G., Sarin, P. S., Reitz, M. S., and Gallo, R. C., *Nature new Biol.*, **240**, 67 (1972).
- ⁶ Gallo, R. C., Sarin, P. S., Smith, R. G., Bobrow, S. N., Sarngadharan, M. G., and Reitz, M. S., in *DNA Replication in vitro* (edit. by Wells, R., and Inman, R.) (University Park Press, Baltimore, in the press).
- ⁷ Gallo, R. C., Sarin, P. S., Sarngadharan, M. G., Smith, R. G., Bobrow, S. N., and Reitz, M. S., in *Proc. Sixth Miles Int. Symp.* (in the press).
- ⁸ Baxt, W., Hehlmann, R., and Spiegelman, S., *Nature new Biol.*, **240**, 72 (1972).
- ⁹ Smith, R. G., and Gallo, R. C., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2879 (1972).
- ¹⁰ Schlom, J., Harter, D. H., Burny, A., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 182 (1972).
- ¹¹ Schlom, J., and Spiegelman, S., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1613 (1971).
- ¹² Gerwin, B. I., Todaro, G. J., Zeve, V., Scolnick, E. M., and Aaronson, S. A., *Nature*, **228**, 435 (1970).
- ¹³ Aaronson, S. A., Parks, W. P., Scolnick, E. M., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 920 (1971).
- ¹⁴ Parks, W. P., Scolnick, E. M., Ross, J., Todaro, G. J., and Aaronson, S. A., *J. Virol.*, **9**, 110 (1972).
- ¹⁵ Watson, K. F., Nowinski, R. C., Yaniv, A., and Spiegelman, S., *J. Virol.*, **10**, 951 (1972).
- ¹⁶ Theilen, G. H., Gould, D., Fowler, M., and Dungworth, D., *J. natn. Cancer Inst.*, **47**, 881 (1971).
- ¹⁷ Wolfe, L. G., Deinhardt, F., Theilen, G. H., Rabin, H., Kawakami, T., and Bustad, L. K., *J. natn. Cancer Inst.*, **47**, 1115 (1971).
- ¹⁸ Kawakami, T., Huff, S. D., Buckley, P. M., Dungworth, D. L., Snyder, S. P., and Gilden, R. V., *Nature new Biol.*, **235**, 170 (1972).
- ¹⁹ Scolnick, E. M., Parks, W. P., and Todaro, G. J., *Science*, **N.Y.**, **177**, 1119 (1972).
- ²⁰ Riddick, D. H., and Gallo, R. C., *Cancer Res.*, **30**, 2484 (1970).
- ²¹ Parks, W. P., Livingston, D. M., Todaro, G. J., Benveniste, R. E., and Scolnick, E. M., *J. exp. Med.*, **137**, 622 (1973).
- ²² Todaro, G. J., Scolnick, E. M., Parks, W. P., Livingston, D. M., and Aaronson, S. A., in *Proc. Sixth Miles Int. Symp.* (in the press).
- ²³ Kang, C.-Y., and Temin, H. M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 1550 (1972).
- ²⁴ Fridlender, B., Fry, M., Bolden, A., and Weissbach, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 452 (1972).
- ²⁵ McCaffrey, R., Smoler, D., and Baltimore, D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 521 (1973).
- ²⁶ Huebner, R., and Todaro, G. J., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 1087 (1969).
- ²⁷ Temin, H. M., *Natn. Cancer Inst. Monogr.*, **17**, 557 (1964).
- ²⁸ Reitz, M., Gillespie, D., Saxinger, W. C., Roberts, M., and Gallo, R. C., *Biochem. biophys. Res. Commun.*, **49**, 1216 (1972).

Table 2 Relative Ability of Anti-virus Polymerase Antibodies to Inhibit the Human Leukaemic Cell Enzyme HL3

Anti-pol serum	Relative inhibitory activity*
Gibbon ape	1-2
Woolly monkey	3-5
Mouse	5-10
Cat	>10
RD114	>20
Avian	>50
Mason-Pfizer	>10

*The concentration of antiserum needed for 30% inhibition of the human leukaemic enzyme activity divided by the antiserum concentration needed to give 30% inhibition of the viral enzyme to which the antibody was prepared. Each antiserum was tested, as in the legend to Fig. 2, at concentrations from 0.6 to 60 µg IgG for ability to inhibit the viral enzyme to which it was prepared.

LETTERS TO NATURE

PHYSICAL SCIENCES

Apollo 17 "Orange Soil" and Meteorite Impact on Liquid Lava

THE "orange soil" from Shorty Crater differs greatly from ordinary lunar soils in that it consists of ~99% 10–300 μm smooth shiny spherules and broken fragments of spherules of transparent orange glass, about 20% of which contain partly crystallized to opaque material. The remaining 1% is chiefly crystalline basalt fragments. Although the colour of the individual orange spherule varies with thickness from yellow-orange to red-brown, all orange glass in our sample (74220, 70; 0.25 g) has a uniform index of refraction (~1.712). By contrast, other lunar soils contain spherules ranging from 1.50 to 1.75. The orange glass is also completely free of bubbles, to the limit of resolution of the light microscope, whereas bubbles are present in many other spherule samples. The spherules generally appear spherical in a normal microscope mount, but when viewed from two directions many are found to be oblate spheroids with axial ratios varying from near 1.00 to as low as 0.42 (Fig. 1a). Some have fissioned during free flight¹ and all stages of the fission process are found, as described for the Apollo 11 samples. Only a few spherules seem to have been distorted by landing while still soft. One notable exception is the occurrence of small spherules of orange glass conforming and adhering to the surface of larger black spherules (Fig. 1b).

Where free of crystals, the orange glass is remarkably uniform in composition from one particle to another (Table 1). The composition is that of an ilmenite-olivine mare basalt, as shown by the CIPW norm.

About 0.2% of spherules >150 μm contain coarse, solid, sharply euhedral, unzoned Fo_{88} olivine crystals as long as 320 μm , embedded in orange glass with smoothly convex to almost circular outer surfaces (Fig. 1c, d). Electron microprobe traces across them reveal neither compositional zoning in the phenocrysts nor gradients in the adjacent glass. Similar olivine occurs as fragments, in part with adhering glass, in the finer size ranges.

The black spherules (and fragments) are believed to have been orange ones that have partly crystallized. They consist chiefly of 1–5 μm laths of olivine of about Fo_{70} (Fig. 1e), decorated by even thinner platelets, of an opaque Fe-Ti phase, possibly epitaxially arrayed. The adjoining glass is depleted in Mg, Fe and Ti, but the gross compositions are similar to that of the orange glass. One 18 μm sphere of nickel iron with 27.1% Ni and 1.84% Co was found in a partly black spherule.

The composition of the orange glass spherules is so similar to some individual spherules from other lunar samples^{2,3} that the differences could only be analytical. But in those other sites spherules constitute a minor component and they vary grossly in composition from one spherule to another. The only other examples of aggregations of homogeneous spherules are two Apollo 11 soil fragments consisting of 90% uniform orange glass⁴, a similar fragment from Apollo 12 consisting of more than 90% dark red, high titanium glass⁵, and the well known clods of uniform green glass spheres from Apollo 15 (ref. 6).

Three other mechanisms have been suggested previously for the origin of the orange soil (and other similar samples): condensation from vapour; spray from volcanic fire fountains; and melting and spray from meteorite impact on the solid lunar crust. Each of these three origins seems to be precluded by one or more of the following ten pieces of evidence.

(1) High speed of rotation during cooling. Very high rates of shear are required to obtain the high rotation rates needed to distort spherules as small as 40 μm into very flattened spheroids (Fig. 1a) against the restoring force of surface tension. We do not believe such high shear rates could be produced in a volcanic fire fountain or on condensation from a vapour. Further, spherules that we have examined from terrestrial fire fountains are generally almost spherical, except for those with attached threads.

Table 1 Electron Microprobe Analyses (Weight Per Cent) of Orange Glass from Sample 74220, 70

	% *	CIPW norm	
SiO ₂	39.5	or 0.5	F- 19.8
Al ₂ O ₃	6.41	ab 4.3	
FeO	22.2	an 15.0	
MgO	14.4	wo 8.4	P- 40.5
CaO	7.13	en 18.5	
Na ₂ O	0.51	fs 13.7	
K ₂ O	0.08	fo 12.3	ol- 22.5
TiO ₂	8.56	fa 10.1	
P ₂ O ₅	0.05	il 16.3	ox- 17.1
MnO	0.32	cm 0.8	
Cr ₂ O ₃	0.52	ap 0.1	
Arithmetic total 99.68		100.0	

* Average of twelve individual spherule analyses (only clear glass spherules, free of crystals, were selected); the chief elements do not vary outside the precision of any individual analysis (1 to 5% of the amount present for elements >5%).

Analyst: Paul W. Weiblen.

Cross⁷ drew an analogy between the lunar spherules and the glass spherules formed incidental to the manufacture of commercial mineral wool insulation, in which a stream of silicate melt is intersected at a large angle by a high velocity steam blast. This process subjects the melt to much greater shear during its acceleration than would occur if the direction of movement of the melt particles and of the accelerating gas were essentially parallel, as in a volcanic explosion or violent fountaining. In this connexion we note that broken fragments of glass threads (Pele's hair) are very scarce in the lunar soils, and we have found none in 74220. We suggest that this lack of Pele's hair probably stems from the bulk of the disaggregation of the melt occurring in the early part of the ejection process, before the temperature can drop sufficiently to raise the viscosity to the point where filaments could form under the mechanical stresses available from rotation⁸.

(2) The olivine phenocrysts. These effectively preclude the first two origins suggested. They must have crystallized before the spherules were formed, and they differ from the olivine laths formed by later crystallization in several important ways: the phenocrysts are unzoned, more magnesian, and much larger than the laths; they are of normal olivine habit and occur in melt that is not depleted in Mg; the phenocryst-

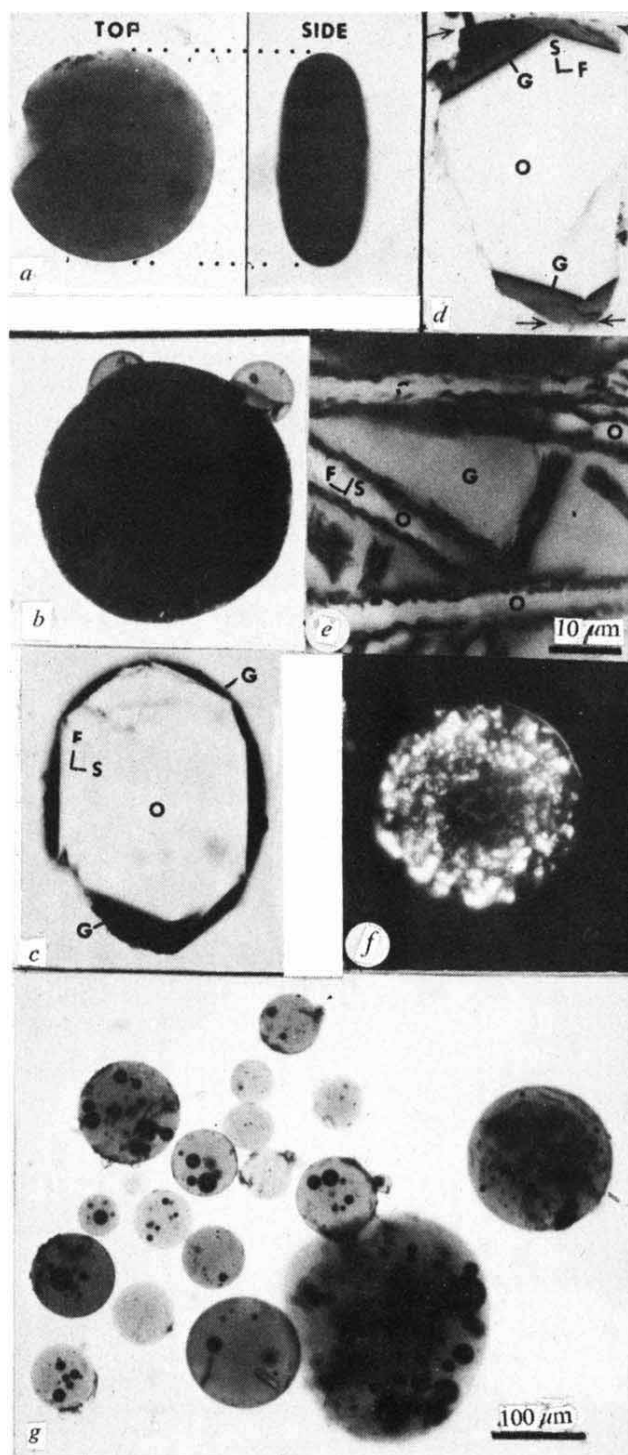


Fig. 1 *a*, Chipped oblate spheroid of diameter 40 μm from sample 74220, in 1.710 oil. Viewed in transmitted red light along short axis (left) and turned on edge (right). *b*, Opaque (crystallized) spherule, 100 μm in diameter, from sample 74220, with two adhering globules of orange glass, suggesting that the black spherule was effectively solid before impacting with the orange ones. Mounted in epoxy resin of $n=1.55$ and viewed in transmitted red light. *c*, Euhedral phenocryst of olivine (O), 145 μm long, with vibration directions indicated, coated with orange glass (G) having a smoothly curved convex surface except where chipped. Sample 74220; transmitted light. *d*, Euhedral phenocryst of olivine (O), maximum dimension 320 μm , with vibration directions indicated, embedded in orange glass (G) having a smoothly convex outer surface where unbroken (arrows). We note the oxide decoration and the tiny projections (overgrowths) at the apices of the crystal. Sample 74220; transmitted light. Black mark in upper left is from electron beam. *e*, Section of spherule from sample 74220 showing blades of olivine (O), up to 5 μm thick, outlined by platelets of opaque Fe-Ti phase at about a 20° angle (epitaxial?) in orange glass (G) depleted in Mg, Fe, and Ti. Transmitted red light. *f*, Orange glass spherule, 68 μm in diameter, from the sample 74220, formerly all glass, shown after cooling in the laboratory at $>500^\circ\text{C}$ s^{-1} . Crossed polars, transmitted light. *g*, Group of basaltic spherules (actually spheres; n about 1.60) from February 22 to 28, 1969, eruption of Alae lava lake, Hawaii, in oil of $n=1.595$. Almost all spherules have gas bubbles, but some have none in this particular plane of focus. Transmitted plain light.

bearing spherules would be more than 80% normative olivine, yet no glass even trending toward these compositions has been found, and no spherules were found with olivine crystals intermediate in size between the phenocrysts and the laths; and only a trace of oxide decoration and overgrowth was found on the phenocrysts, presumably formed during quench (Fig. 1*d*).

(3) High rate of cooling. A melt of this composition can be expected to nucleate and crystallize rapidly—much more rapidly than ordinary terrestrial basalts. To place some crude limits on the cooling rate needed to prevent crystallization, small orange glass spherules were heated in a vacuum in silica capillary tubes and quenched rapidly⁹. From such experiments (Fig. 1*f*) we estimated that cooling rates in excess of $1,000^\circ\text{C s}^{-1}$ were needed to maintain these spherules as glass. Such cooling rates are very likely for minute projectiles ejected in an expanding cloud from an impact event on the Moon, where the individual particle leaves the central source of radiant heat at high velocity; they seem almost impossible to achieve, however, for any volume of material in a volcanic fire fountain.

(4) Uniformity of composition of glass. This is difficult to explain by an impact on solid rock, which normally produces individual spherules having a range of compositions produced by melting of complete rocks and individual constituent minerals^{2,4,5}. Impact on a liquid melt would not produce such diversity. It is conceivable that a very large impact could form an adequate volume of homogeneous melt with an ejection trajectory such that it would be deposited without appreciable contamination by other ejecta formed at the same time, but this explanation is negated by the phenocrysts.

(5) Apparent large amount of spherules at the site. Terrestrial basaltic fire fountains do form spherules, but only to a very small extent, even though the samples of them we see are probably concentrated by air current winnowing that could not occur on the Moon.

(6) Absence of coarse blebs of glass of same composition. Terrestrial glassy basaltic pyroclastics consist almost entirely of much coarser material than the orange soil (spatter, bombs, cinder, and so on) and the physical differences in the environment are not likely to change this drastically on the Moon.

(7) Absence of shocked rock debris. Hypervelocity impact cratering studies¹⁰ have shown that about ninety-nine volumes of crushed and shocked rock are formed and ejected for each volume of liquid melt (glass) that is formed and ejected, and that this glass is generally well dispersed through the other coarse debris. Impact into a liquid would probably eject as much if not more material than impact into solid. Thus only 1% of olivine phenocrysts in the ejecta might possibly show signs of shock damage. These might be recognized in a larger aliquot. Contrary to the suggestion of Cross⁷, we believe that this ejected liquid would be subject to continued disaggregation by spinning and fission during flight because of the high rate of shear produced by the original ejection. Such disaggregation would be most effective on the larger masses, where the radial disruptive forces from spinning would be strongest, and the cohesive (surface tension) forces weakest. Presumably this disaggregation continued down to a minimum threshold spherule diameter ($\sim 12 \mu\text{m}$), yielding the surprisingly uniform small particle size found in the orange glass.

(8) Absence of minute iron metal dust in the glass. In impact on solids, glass is only formed in the region of extreme

ine (O), up to 5 μm thick, outlined by platelets of opaque Fe-Ti phase at about a 20° angle (epitaxial?) in orange glass (G) depleted in Mg, Fe, and Ti. Transmitted red light. *f*, Orange glass spherule, 68 μm in diameter, from the sample 74220, formerly all glass, shown after cooling in the laboratory at $>500^\circ\text{C}$ s^{-1} . Crossed polars, transmitted light. *g*, Group of basaltic spherules (actually spheres; n about 1.60) from February 22 to 28, 1969, eruption of Alae lava lake, Hawaii, in oil of $n=1.595$. Almost all spherules have gas bubbles, but some have none in this particular plane of focus. Transmitted plain light.

temperatures, and hence reduction of iron, as from solar wind hydrogen¹¹, is common in such glass. Impact into lava would be expected to eject large volumes of liquid, most of which would suffer almost no additional heating from impact.

(9) Absence of hypervelocity "zap pits" on the orange spherule surfaces. This was offered as evidence against an impact origin, because such pits are abundant in other impact glass spherules. It is to be expected, however, if the bulk of the orange spherules originated from liquid ejected at a distance from the centre of the original impact crater, where secondary impacts forming zap pits were less abundant. Also, the great age of the orange soil reported by O. Schaeffer of SUNY, and by other groups ($\sim 3.7 \times 10^9$ yr), the short cosmic-ray exposure age (~ 30 m.y.), the field data, and the almost complete lack of extraneous material indicate that the orange soil was buried deep enough to be effectively shielded from later surface impact mixing—and the expected concomitant micrometeorite flux—for most of its life. Seemingly, it was then exposed by the relatively "recent" impact event forming Shorty Crater.

Agrell *et al.*¹² and McKay *et al.*¹³, reporting on the somewhat similar Apollo 15 green glass spherules, suggest that the complex adhesions and minute low velocity impact pits of one globule on another of the same composition are evidence against an impact origin for the green glass (and, by analogy, for the orange glass too; see Fig. 1b). But the expanding cloud of liquid droplets from impact on liquid lava would probably have a much greater density of glass droplets than would be produced by an impact on solid rock, and hence the probabilities of secondary "impact" from differing velocities or trajectories during flight would be much greater.

The black spherules might arise from slower cooling rates caused by slightly different trajectories. Alternatively, they might arise from thermal stratification of the lava lake before impact. The occasional crystalline basalt fragment in the orange soil might be part of a solidified crust.

(10) Complete lack of bubbles. This might seem trivial, but we believe it effectively eliminates the second postulated origin. Terrestrial volcanic fire fountaining is generally believed to be driven by rapid gas bubble nucleation and expansion. This in turn requires supersaturation of volatiles in the liquid, and the resulting spherules almost always contain gas bubbles (Fig. 1g). Some of the smallest spherules from Hawaii (volume $\sim 30 \mu\text{m}^3$) are bubble-free, as expected from the short diffusion distances for loss of volatiles at the surface, and because nucleation probabilities become predominant in such small volumes⁹. By contrast, no bubble was found in any of our much larger orange glass spherules. This also implies that the lunar lava impacted was extremely low in volatiles. In contrast, lunar glasses formed by impact fusion of lunar soils contain many bubbles, probably from outgassing of solar wind constituents^{11,14}.

One objection that has been raised to our hypothesis is that the exact composition of the orange glass (and the Apollo 15 green glass also) is not found among the crystalline igneous rocks returned from the various lunar sites. This could be simply a sampling problem, and there are Apollo 11 and 17 basalts that do not differ greatly in principal constituents from the orange glass. The problems involved in explaining the unusual trace element assemblage in the orange glass¹⁵ are just as applicable to any proposed origin for the materials that comprise the orange soil.

We have no evidence on the magnitude of the lunar impact event that formed the orange soil, but if the orange spherules from the other lunar sites are from the same impact, the event was probably large and distant. The unusual time coincidence involved in having a meteorite impact on liquid lava is more apparent than real, in view of the convincing evidence that there was a cataclysmic series of events on the Moon at about 3.7×10^9 yr ago, when this sample was formed. In addition, a crusted lava flow or lake is just as permissible by our hypothesis, thus allowing a much longer time span (after extrusion

of the lava) during which the impact event that formed the orange glass could occur. Such impacts onto crusted lava should yield craters with very different characteristics than those formed in solidified lava flows, and the maria should be examined for evidence of such craters.

EDWIN ROEDDER
PAUL W. WEIBLEN

US Geological Survey,
Washington DC 20244
and
Department of Geology,
University of Minnesota,
Minneapolis, Minnesota 55455

Received March 30, 1973.

- ¹ Fulchignoni, M., Funicioello, R., Taddeucci, A., and Trigila, R., *Proc. Second Lunar Sci. Conf.*, 1, 937 (MIT, Cambridge, 1971).
- ² Chao, E. C. T., Boreman, J. A., Minkin, J. A., and James, O. B., *J. geophys. Res.*, 75, 7456 (1970).
- ³ Ware, N. G., and Lovering, J. F., *Science*, N.Y., 167, 519 (1970).
- ⁴ Wood, J. A., Marvin, U. B., Powell, B. N., and Dickey, jun., J. S., *Smithsonian Astrophysical Observatory Spec. Rep.* 307 (1970).
- ⁵ Wood, J. A., *et al.*, *Smithsonian Astrophysical Observatory Spec. Rep.* 333, 165 (1971).
- ⁶ Chamberlin, J. W., and Watkins, C., *The Apollo 15 Lunar Samples* (Lunar Science Institute, Houston, 1972).
- ⁷ Cross, C. A., *Nature*, 233, 185 (1971).
- ⁸ Kingery, W. D., *Property Measurements at High Temperatures* (Wiley, New York, 1959).
- ⁹ Roedder, E., *Proc. IMA-IGOD Meetings 1970, IMA Vol.*, Min. Soc. Japan Spec. Pap., 1, 5 (1971).
- ¹⁰ Short, N. M., *Modern Geol.*, 1, 81 (1969).
- ¹¹ Grant, R. W., Housley, R. M., and Paton, N. E., *Lunar Science IV*, 315 (Lunar Science Institute, Houston, 1972).
- ¹² Agrell, S. O., Agrell, J. E., Arnold, A. R., and Bristol, C. C., *Lunar Science IV*, 12 (Lunar Science Institute, Houston, 1972).
- ¹³ McKay, D. S., Clanton, U. S., and Ladle, G. H., *Lunar Science IV*, 484 (Lunar Science Institute, Houston, 1972).
- ¹⁴ Roedder, E., and Weiblen, P. W., *Proc. Apollo 11 Lunar Sci. Conf.*, 1, 833 (Pergamon, New York, 1970).
- ¹⁵ Brown, G. M., Holland, J. G., and Peckett, A., *Nature*, 242, 515 (1973).

Long-term Behaviour of Hercules X-1

HER X-1 was observed with the Copernicus satellite X-ray detectors on June 5 this year. This date was chosen because it was predicted to be within an "extended low" of the 35-day cycle reported by Giacconi *et al.*¹. The purpose of the observation was to search for the steady soft component, hypothesized^{2,3} to account for the persistence through the "extended lows" of the ~ 1.5 mag amplitude, 1.7 d period, optical variations which are generally attributed to heating of one face of the companion star by the X-ray source. We report here the unexpected result that Her X-1 was detected in the 4 to 12 keV channel (overlapping with the energy range covered by Uhuru), at an intensity comparable with the previously measured "high state" intensity.

In Fig. 1 we show the counting rate in the 4 to 12 keV channel (after subtraction of background) for a 16 h period on June 5, 1973, from 6 h 18 min UT to 22 h 12 min UT. For 1.75 h the telescope was pointing at Her X-1: both before and after that time it was directed elsewhere. There is an excess count rate when pointing towards Her X-1, the mean of which is 11.2 s.d. above zero. Such an effect has never been observed when looking at non-X-ray objects. It would seem an extraordinary coincidence if the background subtraction method failed by this significant factor just when the instruments were directed at Her X-1. This intensity cannot be related directly to Uhuru counts because of uncertainties in source spectrum, quantum efficiencies, and often factors. We estimate, however, that it corresponds to 30 to 100 Uhuru counts s^{-1} and that it is close to the maximum high state intensity of ~ 100 counts (well above the count rate of $\lesssim 10$ counts reported through the

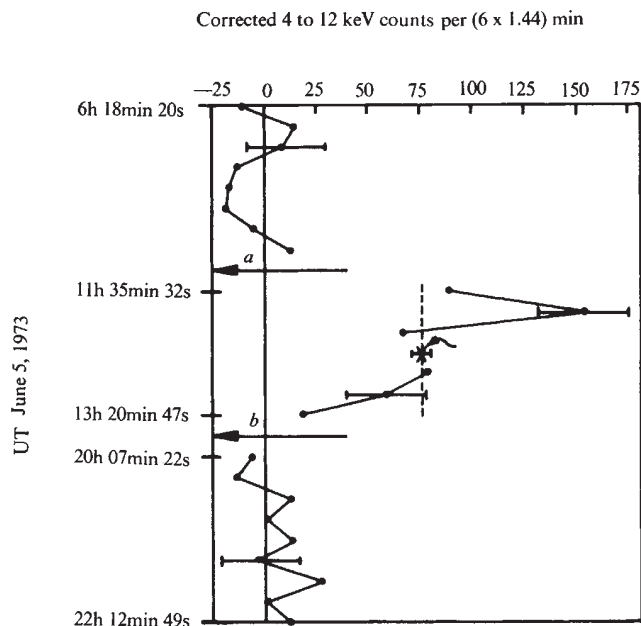


Fig. 1 Corrected 4 to 12 keV counts accumulated per six integration periods displayed against time. The gaps in the time sequence are due to Earth occultations, South Atlantic anomaly passes and time spent in viewing other X-ray sources. $\times$ $\times$ $\times$, Mean Her X-1 count; a, slew to Her X-1; b, slew from Her X-1.

“extended low”). The latest definitely observed onset of the high state reported by the Uhuru group occurred on July 3, 1972. For June 5, 1973, to fall in a high state as well, the period of the cycle would have to be <33.8 d or >36.7 d. Further, Her X-1 was also observed from Copernicus on May 16, 1973, 3 d after the turn on predicted by a 34.88 d cycle, at a comparable intensity. These two Copernicus results are inconsistent with the ≤ 12 d duration quoted for the “high state”. On neither of these occurrences was the X-ray source predicted to be eclipsed by its companions. A subsequent observation was, however, made on June 6 (13 to 14 h after that shown in Fig. 1) when the X-ray source should have been eclipsed and no significant excess flux was recorded.

It therefore seems that the so-called “extended lows” may be a less well defined, or less strictly periodic, feature of Her X-1 than has been claimed.

Simultaneous observations with the 0.5 to 1.5 keV and 1.5 to 4.2 keV telescopes provide upper limits of ~ 1 count per 1.44 min integration period. A count rate of this order at 1 keV corresponds to a luminosity in this energy range of $\leq 2 \times 10^{34} \exp(\tau)$ erg s^{-1} keV $^{-1}$, where τ is the optical depth and we assume a distance of 5.8 kpc.

These observations thus provide no evidence for a steady soft X-ray flux strong enough to heat the companion. There is, however, no pressing need to invoke such a flux until simultaneous X-ray and optical observations reveal occasions when the star is being heated but there are no X-rays >2 keV. We note that the published optical data were all obtained at times when the “low state” was inferred (by extrapolating previous behaviour) rather than being directly observed.

We thank Professor R. L. F. Boyd and his colleagues for use of the spacecraft and the RSRs staff on the project at the Goddard Space Flight Center for their help. J. E. P. acknowledges an SRC studentship.

A. C. FABIAN

Mullard Space Science Laboratory,
University College, London

J. E. PRINGLE
M. J. REES

Astronomy Centre,
University of Sussex

Received June 29, 1973.

- ¹ Giacconi, R., Gursky, H., Kellogg, E., Levinson, R., Schreier, E., and Tananbaum, H., *Astrophys. J.* (in the press).
- ² Avni, Y., Bahcall, J. N., Joss, P. C., Bahcall, N. A., Lamb, F. K., Pettick, C. J., and Pines, D., *Astrophys. J.* (in the press).
- ³ Pringle, J. E., *Nature phys. Sci.*, **243**, 90 (1973).

Recurrence of Seismic Migrations along the Central California Segment of the San Andreas Fault System

VERIFICATIONS of tectonic concepts¹ concerning seafloor spreading are emerging in a manner that has direct bearing on earthquake prediction. Although the gross pattern of worldwide seismicity contributed to the formulation of the plate tectonic hypothesis, it is the space-time characteristics of this seismicity that may contribute more toward understanding the kinematics and dynamics of the driving mechanism long speculated to originate in the mantle. If the lithosphere is composed of plates that move essentially as rigid bodies, then there should be seismic edge effects associated with this movement. It is these interplate effects, especially seismic migration patterns, that we discuss here. The unidirectional propagation at constant velocity (80 km yr^{-1} east to west) for earthquakes ($M \geq 7.2$) on the Antolian fault for the period 1939 to 1956 (ref. 2) is one of the earliest observations of such a phenomenon. Similar studies^{3,4} of the Alaska Aleutian seismic zone and certain regions of the west coast of South America suggest unidirectional and recurring migrations of earthquakes ($M \geq 7.7$) occur in these areas. Between these two regions along the great transform faults of the west coast of North America, there is some evidence⁵ for unidirectional, constant velocity and recurrent migration of great earthquakes. The small population of earthquakes ($M > 7.2$) in Savage's investigation⁵ indicates a large spatial gap along the San Andreas system in central California from 1830 to 1970. Previous work on the seismicity of this gap in central California indicates that the recurrence curves remain relatively constant, independent of large earthquakes, for periods up to a century⁶. Recurrence intervals for earthquakes along the San Andreas Fault have been calculated empirically by Wallace⁷ on the basis of geological evidence, surface measurements and assumptions restricted to the surficial seismic layer. Here we examine the evidence for recurrence of seismic migrations along the San Andreas fault system of central California for earthquakes of magnitude $M \geq 5$.

Figure 1 is an index map of the region studied. The two parallel lines mark the bounds of the area included in our study. The southerly extent of recent microearthquake data (1969–1971) also shown in Fig. 1 delineates the approximate position and strike of the San Andreas fault. This activity terminates abruptly near latitude 37° and continues northerly on the east side of the San Francisco Bay along the Hayward-Calaveras fault system. The lineation of activity that links the San Andreas fault to the Hayward-Calaveras faults near latitude 37° is the Sargent fault. These faults are referred to collectively here as the San Andreas fault system. The data (Table 1) used in Fig. 2 are a subset of data taken from two sources^{8,9}. Figure 2 is a space-time plot of earthquakes ($M \geq 5$) within 30 km of the San Andreas fault between latitudes $35^\circ 30'$ and $38^\circ 30'$ in the period 1930 to 1972. The upper and lower bases lie within the landward extensions of the Pioneer and Murray fracture zones, respectively. These latitude bounds were set to exclude the contrasting tectonic regimes of the Transverse Range Province and the Mendocino escarpment. The most outstanding characteristic of the plot is the envelope of slope 3.3 km yr^{-1} that separates a space-time area of earlier seismic inactivity from one of prevalent seismicity. We consider the aseismic triangle lying above the envelope significant because of the level of the magnitude cutoff ($M \geq 5$), and because the principal seismo-

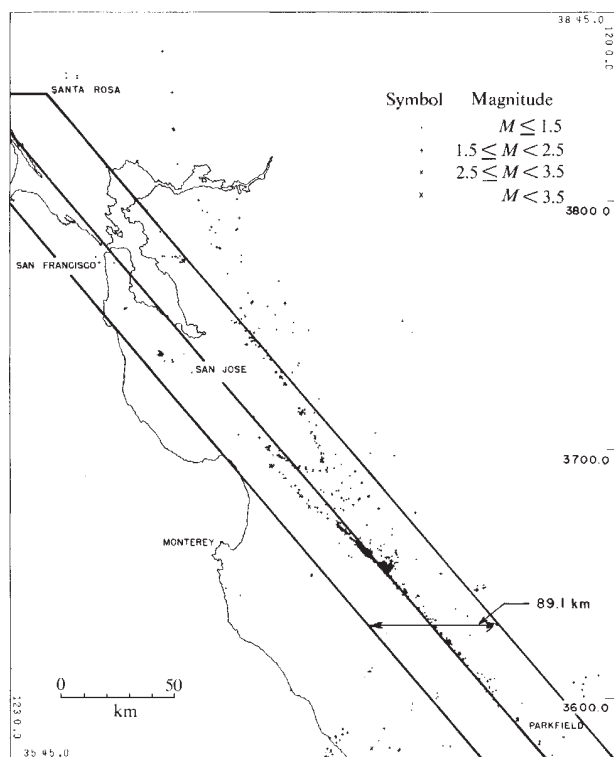


Fig. 1 Index map of region studied. Earthquakes in this seismically active area of central California delineate the San Andreas and Hayward-Calaveras fault systems. Events ($M \geq 5.0$) used in seismic migration study fall within 30 km of the San Andreas fault between latitudes $35^\circ 30' \text{ N}$ and $38^\circ 30' \text{ N}$ and cover the period 1930-1972.

graph stations include Berkeley ($37^\circ 52' \text{ N}$, $122^\circ 15.6' \text{ W}$) and Mt Hamilton ($37^\circ 20.5' \text{ N}$, $122^\circ 38.5' \text{ W}$) which have been well situated to record such events at least since 1930. Four recurrent cycles were also derived by fitting the remaining data to lines parallel to the envelope. This fitting procedure provided a basis for comparison and interpretation of results derived by Savage⁵ which cover more of the fault with but few earthquakes ($M > 7.2$).

Table 1 Earthquakes greater than Magnitude 5 within 30 km of the San Andreas Fault between Latitudes $35^\circ 30'$ and $38^\circ 30'$

Year	Month	Day	Latitude	Longitude	Magnitude
1934	6	5	35 48	120 20	5.0
1934	6	8	35 48	120 20	5.0
1934	6	8	35 48	120 20	6.0
1934	12	24	35 56	120 29	5.0
1938	9	27	36 18	120 54	5.0
1939	6	24	36 24	121 0	5.5
1939	12	28	35 48	120 20	5.0
1947	2	5	36 14	120 39	5.0
1949	3	9	37 1	121 29	5.2
1951	7	29	36 35	121 11	5.0
1954	4	25	36 56	121 41	5.3
1955	9	5	37 22	121 47	5.5
1955	10	24	37 58	122 3	5.4
1955	11	2	36 0	120 55	5.2
1956	11	16	35 57	120 28	5.0
1957	3	22	37 40	122 29	5.3
1959	3	2	36 59	121 36	5.3
1960	1	20	36 47	121 26	5.0
1961	4	9	36 41	121 18	5.6
1961	4	9	36 42	121 18	5.5
1963	9	14	36 52	121 38	5.4
1964	11	16	37 3	121 41	5.0
1966	6	28	35 54	120 32	5.6
1967	12	18	37 0	121 47	5.3
1969	10	2	38 28	122 41	5.6
1969	10	2	38 27	122 41	5.7
1972	2	24	36 34	121 12	5.0

Savage assumes that deformation across the fault zone is governed by a creep law. This process is described as a flow of edge dislocations along the transform fault from a source associated with injection of new material from a spreading centre (East Pacific Rise) to a sink associated with a descending plate (Aleutian trench). The creep wave associated with this process, once initiated, propagates at a constant velocity towards the sink. Earthquakes are thought to be manifestations of the creep wave but occur only along segments of the fault where the dislocation density has increased beyond the threshold for failure. The migration velocity (estimated to be of the order of km yr^{-1}) need not be similar to the velocities of shallow slip and creep waves (km d^{-1}) in the surficial seismic layer reported by King *et al.*¹⁰ or the velocity associated with seafloor spreading which is of the order of cm yr^{-1} (ref. 11). The recurrence interval of these waves is probably controlled chiefly by the boundary conditions at the source and sink. This model also suggests that the waves of different velocities will pass by a point at any given time. Savage's data suggest for events $M > 7.2$ a south-north migration velocity of 60 km yr^{-1} and our data suggest for events $M \geq 5.0$ a south-north migration velocity of 3 km yr^{-1} . The inference is tempting that a continuum of velocity exists such that lesser magnitude events infer waves of proportionally lower velocity. As a partial test of this hypothesis, micro-earthquake data covering the period 1969-1972 from Lee *et al.*¹²⁻¹⁴ were also processed in a manner identical to that for events $M > 5$. No significant pattern of recurrent migration emerges for microearthquakes during the period 1969-1971 and/or a fault length greater than 50 km. Only when smaller segments (50 km or less) of certain of the principal faults in central California are examined are progressions of such small events observed¹⁵.

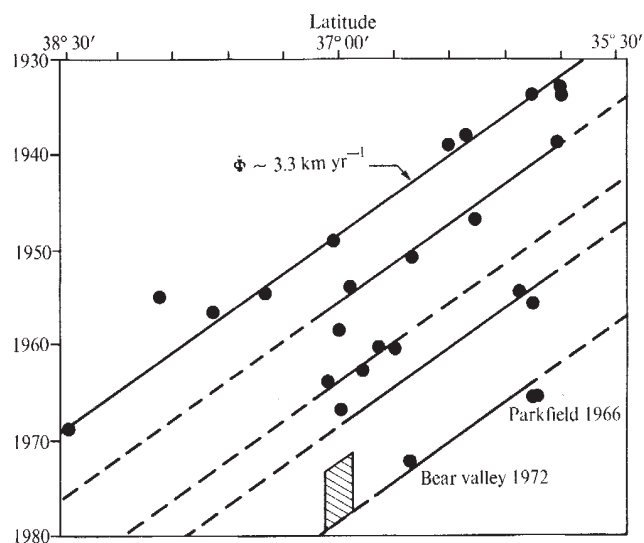


Fig. 2 Space-time plot of earthquakes studied. Based on evidence of seismic migration pattern and latitude of maximum recurrence of events, it is predicted that the next earthquake of magnitude $M \geq 5.0$ will occur within the hatched area on the plot.

The apparent recurrent cycles of south to north migration of earthquakes $M \geq 5.0$ are void of seismic activity in the region north of latitude 37° . This latitude also coincides with the abrupt termination of significant microearthquake activity¹⁶ and fault creep^{17,18} along the San Andreas fault. The most recent wave seems to be associated with the Parkfield-Cholame earthquake of 1966 (ref. 19) and has propagated at least as far north as the Bear Valley earthquake of February 1972 (labelled on Fig. 1).

The projection of the most recent seismic migration wave to the latitude of maximum recurrence of earthquakes provides the basis for setting the space-time bounds on the next earth-

quake greater than magnitude 5. The most likely location for the next earthquake $M \geq 5.0$ within this 350 km segment of the San Andreas system is predicted to occur near latitude 37° before 1978.

M. D. WOOD
S. S. ALLEN

National Center for Earthquake Research,
US Geological Survey,
Menlo Park, California 94025

Received April 18, 1973.

- ¹ Isacks, B., Oliver, J., and Sykes, L. R., *J. geophys. Res.*, **73**, 8555 (1968).
- ² Mogi, K., *Bull. Earthquake Res. Inst.*, **46**, 53 (1968).
- ³ Kelleher, J. A., *J. geophys. Res.*, **75**, 5745 (1970).
- ⁴ Kelleher, J. A., *J. geophys. Res.*, **77**, 2087 (1972).
- ⁵ Savage, J. C., *J. geophys. Res.*, **76**, 1954 (1971).
- ⁶ Ryall, A., Slemmons, D. B., and Gedney, L. D., *Bull. seism. Soc. Am.*, **56**, 1105 (1966).
- ⁷ Wallace, R. E., *Bull. geol. Soc. Am.*, **81**, 2875 (1970).
- ⁸ *Crustal Strain and Fault Movement Investigation*, Bull. 116-2 (California Department of Water Research, 1964).
- ⁹ Bolt, B. A., and Miller, R. D., *Bull. seism. Soc. Am.*, **61**, 1831 (1971).
- ¹⁰ King, C. Y., Nason, R. D., and Tocher, D., *Phil. Trans. R. Soc.* (in the press).
- ¹¹ Atwater, T., *Bull. geol. Soc. Am.*, **81**, 3513 (1970).
- ¹² Lee, W. H. K., Roller, J. C., Bauer, P. C., and Johnson, J. D., *Catalog of Earthquakes along the San Andreas Fault System in Central California for the Year 1969* (US Geological Survey, Open-File Report, 1972).
- ¹³ Lee, W. H. K., Roller, J. C., Meagher, K. L., and Bennett, R. E., *Catalog of Earthquakes along the San Andreas Fault System in Central California for the Year 1970* (US Geological Survey, Open-File Report, 1972).
- ¹⁴ Lee, W. H. K., Meagher, K. L., Bennett, R. E., and Matamoros, E. E., *Catalog of Earthquakes along the San Andreas Fault System in California for the Year 1971* (US Geological Survey, Open-File Report, 1972).
- ¹⁵ Wood, M. D., *Geol. Soc. Am. Abs.*, **4**, 262 (1972).
- ¹⁶ Eaton, J. P., Lee, W. H. K., and Pakiser, L. C., *Tectonophysics*, **9**, 258 (1970).
- ¹⁷ Nason, R. D., *R. Soc. N.Z. Bull.*, **9**, 181 (1971).
- ¹⁸ Savage, J. C., and Burford, R. O., *J. geophys. Res.*, **78**, 832 (1973).
- ¹⁹ Eaton, J. P., O'Neill, M. E., and Murdock, J. N., *Bull. seism. Soc. Am.*, **60**, 1151 (1970).

Chemistry and Morphology of Precambrian Microorganisms

THE relatively unmetamorphosed sedimentary rocks of the Onverwacht Group, the lowest part of the Swaziland Sequence, may contain the oldest microfossils on Earth. The group is composed chiefly of lavas whose composition changes upwards from ultramafic through mafic to predominantly felsic with pyroclastics. The 17,000 m thick sequence includes some thin cherts and limestones together with small amounts of shale (Table 1). In the Lower Onverwacht, the cherts occur chiefly as intercalations, sometimes of wide lateral extent and sometimes lensoid, between the extrusives. Cherts are also found in the Upper Onverwacht in association with limestones and shales.

Occurrences of microstructures from the Upper Onverwacht Swartkoppie Formation have been reported previously¹⁻⁵. Here we provide the first description of microorganisms from the Kromberg and Theespruit Formations. The microstructures reported⁶⁻⁸ from the succeeding Fig Tree Series (3.1×10^9 yr old) are generally accepted as biogenic, but Engel *et al.*¹ have expressed strong reservations about the biogenic origin of the Onverwacht material. Their "acceptable criteria" for biogenicity (after Cloud and Licari⁹) require that the microstructures fall within a narrow size range, possess morphological complexity equivalent to that of living organisms, and have little resemblance to purely inorganic structures. Such criteria are, however,

of limited value, and there is little to be gained from rigid, narrow, concepts in the study of Precambrian microorganisms.

The microfossils from the Fig Tree Group possess simple morphologies, yet no serious doubts have been cast upon their biogenic origin, and it is difficult to understand why the structures in the Onverwacht Group should be considered less likely to be biogenic.

We believe that the most useful way of assessing the possible biogenicity of a microstructure is by relating morphology and chemistry^{5,10}. Because it is at present impractical to analyse chemically single microstructures, the insoluble organic matter was isolated and subjected to both chemical and morphological examination. Thin chips or slices of rock (cut with a diamond saw and then washed carefully with distilled water) were etched in 20% hydrofluoric acid. The etched face was air dried and examined. The released organic residue was also prepared for examination. The morphological examination was carried out using the scanning electron microscope, as well as the light microscope favoured by others¹⁻³.

Table 1 Stratigraphy of the Onverwacht Group, Transvaal, South Africa (Modified after Refs 19, 23)

	Formation	Thickness (m)	Lithology
Upper Onverwacht	Swartkoppie	1,000	Felsic lavas and pyroclastics, some mafic material. Sedimentary intercalations of chert, limestone and shale.
	Kromberg	2,100	Mafic lavas and pyroclastics, some felsic horizons and some intercalated sediment.
	Hoogenoeg	5,300	Tholeiites, felsic lavas and pyroclastics. Chert layers more frequent towards top.
	Middle Marker Horizon	3,355 $\times$ 10 ⁹ yr	Chert, limestone and shale.
	Lower Onverwacht		
	Komati	3,800	Ultramafic and mafic lavas.
	Theespruit	2,100	Mafic lavas, felsic tuffs. Some sediment in upper part. Chert, limestone and shale.
	Sandspruit	2,600	Ultramafic and mafic lavas.

In the Onverwacht Group there are two morphologically simple types of microorganism: spheroids, similar to those previously described¹⁻⁵ in the younger Onverwacht rocks; and filaments. The former are the most abundant and contrary to some earlier results¹⁻³ the size distribution in a single sample tends to fall within a narrow range. The Lower Onverwacht spheroids (Fig. 1a) examined from the Theespruit Formation are not common and are much smaller (7 to 10 μ m diameter) than those microorganisms present in the Upper Onverwacht.

The microorganisms present in the Upper Onverwacht Kromberg (Fig. 1b) and Swartkoppie (Fig. 1c) Formations are larger (15 to 20 μ m diameter), much more abundant and not infrequently found associated in pairs (Fig. 1d). This latter phenomenon, while not perhaps being absolutely conclusive, does support the biogenic origin of the microstructures.

Filamentous bodies have been found *in situ* in the etched chert of the Theespruit (Fig. 1e), Kromberg (Fig. 1f) and Swartkoppie (Fig. 1g and h) Formations. The filaments have diameters of about 4-8 μ m and may be up to 20 μ m long. It seems likely that they have been previously unnoticed because either they are rare and thus difficult to find in thin section (Engel *et al.*¹ mention the patience required to discover even the relatively abundant spheroids), or they are probably destroyed during the pulverization used in conventional extraction techniques.

Because of the occurrence of the organic filaments and the paired spheroids, we suggest that these microstructures are undoubtedly the remains of living organisms, although it is not possible at this stage to deduce their biological affinities.

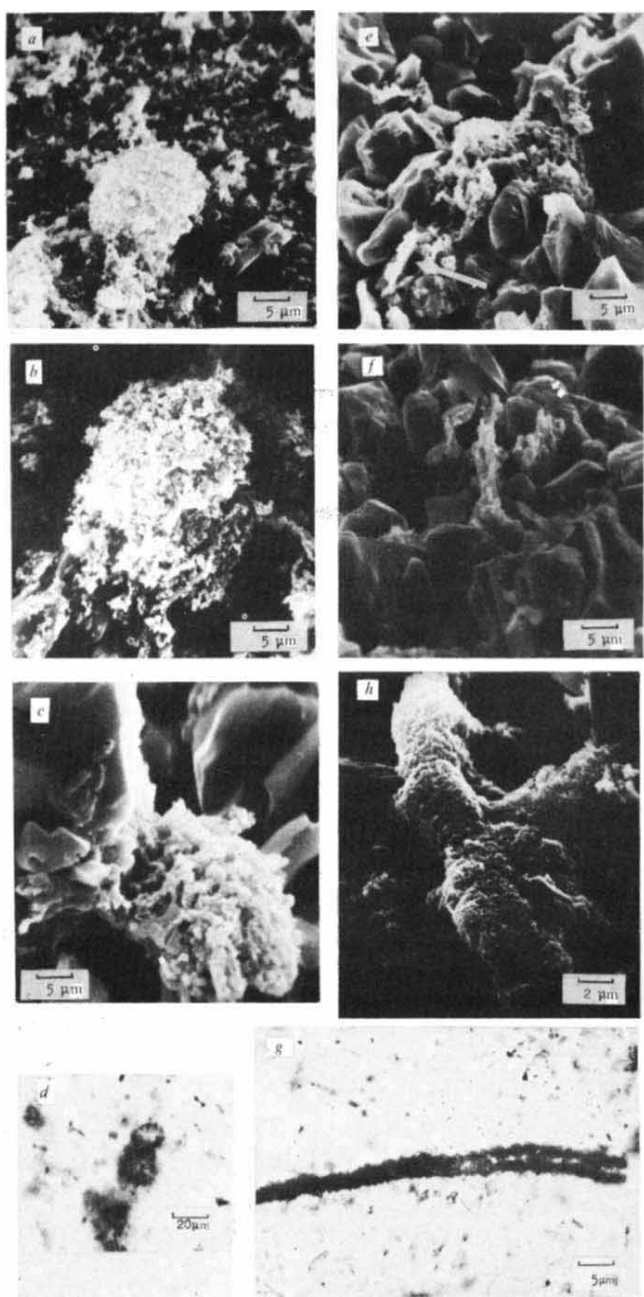


Fig. 1 *a*, Sphere from Theespruit Formation; *b*, sphere from Kromberg Formation; *c*, sphere from Swartkoppie Formation—*in situ* in etched chert; *d*, optical thin section of Swartkoppie thin section; *e*, filament (arrow indicates position)—*in situ* in etched chert of Theespruit Formation; end shows filament is hollow; *f*, filament *in situ* in etched chert of Kromberg Formation; end shows filament is hollow; *g*, filament from Swartkoppie Formation; *h*, filament from Swartkoppie.

The extracted insoluble organic matter was also subjected to ozonolysis. The degradation products were found to be similar to those obtained from other sedimentary insoluble organic matter^{10–12}, and also to the ozonolysis products of various sporopollenins^{4,11,13–15}. Thus the Onverwacht insoluble organic matter is, if not identical with, at least related to, the naturally occurring polymeric material, sporopollenin, of known biogenic origin.

One apparent difficulty in determining whether or not the Onverwacht microorganisms are biological in origin is that chemical studies of the insoluble organic matter give degradation products which suggest a more aromatic original composition than that found in other Precambrian kerogens. Nagy and Nagy³ suggest that because aromatic compounds in coal

and lignite are used to infer the presence of lignin, and because there is no record of lignin in Precambrian rocks, "it is difficult to envisage other biochemicals which could be present in sufficient quantities to account for such a large accumulation of aromatic matter".

But sporopollenins, when heated, very readily give aromatic compounds, including methyl and dimethyl naphthalene, together with compounds such as ionene. The resulting specific mixture of those compounds is virtually identical to that produced from most metamorphosed sedimentary kerogens^{10–13,15}. Potonie and Rehne¹⁶ showed that aromatic compounds are present in fossil sporopollenin. It is therefore unnecessary to postulate a lignin precursor for the aromatic compounds in the Onverwacht Group.

Although the Onverwacht rocks are relatively unmetamorphosed, they have been heavily folded and have suffered low grade metamorphism. This need not imply any very great rise in temperature (Walls, personal communication, has examined some of the carbonate rocks from the Swartkoppie Formation and concludes that they have not been heated above about 200°C), but optically the organic matter is dark and opaque, suggesting some degree of coalification (carbonization). Because the organic matter occurs in sediments intercalated in volcanic sequences considerable local heating effects resulting from extrusion of lavas on to pre-existing sediments are probably responsible for the dark colour of the microfossils. Rye (personal communication) has observed an increase in colour density and a change in colour from light yellow to brown, and finally to black, in organic matter incorporated in the interbasaltic Tertiary sediments from the Scottish Western Isles.

The thermal history of the Onverwacht Group is also important in considering the values of the stable carbon isotopes of the insoluble organic matter. The $\delta^{13}\text{C}$ values for the Upper Onverwacht samples^{12,17} are similar to those found in geologically younger, photosynthetically produced organic matter. The isotope values for the Theespruit Formation samples are, however, anomalously high, and Oehler *et al.*¹⁷ therefore suggest that the organic matter from the Lower and Upper Onverwacht samples may have differing origins. They note a resemblance between the Theespruit $\delta^{13}\text{C}$ values and those recorded from carbonaceous chondrites, and they further suggest that the anomalous values may indicate the presence of non-photosynthetic organisms.

But Silverman¹⁸ showed that heating organic matter results in an evolution of low molecular weight hydrocarbons, such as methane and ethane. The preferential release of ^{13}C in these compounds produces a higher $\delta^{13}\text{C}$ ratio in the residue. Thus, both the anomalous $\delta^{13}\text{C}$ ratio and the aromaticity of the organic matter can be explained by thermal action. This would be more intense in the Theespruit Formation than in the younger Onverwacht Formations, because the Theespruit Formation is a predominantly mafic volcanic sequence, whereas the other formations contain larger amounts of sediment, and more felsic volcanics extruded at lower temperatures.

Further evidence for the biological origin of the Onverwacht Group microstructures can be adduced from the depositional environment of the sediments. These were laid down on a surface of pillow lavas and other subaqueous volcanics. Although some of the sediments are laterally extensive, most occur in pockets in the surface of the lavas¹⁹.

The juvenile water that accompanies present day volcanic activity, both at the time of eruption and during the later fumarole stage, is extremely rich in plant nutrients. The hot springs associated with late Tertiary volcanic activity in the Rocky Mountains (Yellowstone National Park^{20–22}) contain a vast and varied microflora. Blue-green algae and bacteria are particularly common and occur in various micro-environments dependent upon pH, temperature and nutritional factors. Filamentous, rod-like and spheroidal microorganisms occur in waters almost at boiling point. Similar conditions may have existed during formation of the Onverwacht sediments, with organisms that could tolerate high temperatures (up to 100°C)

living in shallow depressions on the surface of hot lavas that, by analogy with present day extrusives, probably maintained high temperatures for considerable periods of time, although the Onverwacht lavas, unlike the Yellowstone examples, were subaqueously extruded. In the recent examples, the waters are charged with juvenile carbon dioxide, phosphorus, sulphur and nitrogen.

Walter²¹ recently suggested a hot springs analogue for the depositional environment of the Gunflint Iron Formation of the Lake Superior region, suggesting that a fossilized geyserite might explain many of the features of the Gunflint siliceous stromatolites. We suggest that a similar analogue is even more appropriate for the Onverwacht sediments, for they occur within a demonstrably volcanic sequence. Such environments would favour the production of small, random, pockets of microorganisms, whose distribution would be controlled by proximity to the hot springs that produced exceptionally favourable nutritive conditions. We thus infer the presence of photosynthesizing microorganisms in the Lower Onverwacht Theespruit Formation (older than 3.355×10^9 yr, ref. 23) and in the Upper Onverwacht Kromberg and Swartkoppie Formations.

We thank Dr Morris Viljoen (Consolidated Investment Company, Johannesburg, South Africa) for detailed information and for collecting the various Onverwacht Group samples.

J. BROOKS*

Exploration and Production,
Research Division,
BP Research Centre,
Sunbury-on-Thames

M. D. MUIR

Geology Department,
Royal School of Mines,
Imperial College,
London SW7 2BP

G. SHAW

School of Chemistry,
University of Bradford,
Bradford 7, Yorkshire

Received January 18; revised March 12, 1973.

* Present address: H. Foster & Co. Ltd, Aire Place Mills, Kirkstall Road, Leeds LS3 1JL.

- ¹ Engel, A. E. J., Nagy, B., Nagy, L. A., Engel, C. G., Kremp, G. O. W., and Drew, C. M., *Science, N.Y.*, **161**, 1005 (1968).
- ² Nagy, B., and Nagy, L. A., in *Advances in Organic Geochemistry* (edit. by Schrenck, P. A., and Havenaar, I.), 208 (Pergamon, Oxford, 1968).
- ³ Nagy, B., and Nagy, L. A., *Nature*, **223**, 1226 (1969).
- ⁴ Brooks, J., and Shaw, G., *Grana*, **11**, 1 (1971).
- ⁵ Brooks, J., and Muir, M. D., *Grana*, **11**, 9 (1971).
- ⁶ Barghoorn, E. S., and Schopf, J. W., *Science, N.Y.*, **152**, 758 (1966).
- ⁷ Pflug, H. D., *Rev. Palaeobotan. Palynol.*, **5**, 9 (1967).
- ⁸ Schopf, J. W., *Biol. Rev.*, **45**, 319 (1970).
- ⁹ Cloud, P. E., and Licari, G. R., *Proc. Natn. Acad. Sci., U.S.A.*, **61**, 779 (1968).
- ¹⁰ Brooks, J., and Shaw, G., in *Origin and Development of Living Systems* (Academic Press, in the press).
- ¹¹ Brooks, J., and Shaw, G., *Nature*, **220**, 678 (1968).
- ¹² Brooks, J., in *Sporopollenin* (edit. by Brooks, J., Grant, P., Muir, M. D., Shaw, G., and Van Gijzel, P.), 351 (Academic Press, 1971).
- ¹³ Shaw, G., in *Phytochemical Phylogeny* (edit. by Harborne, J. B.), 31 (Academic Press, 1970).
- ¹⁴ Brooks, J., and Shaw, G., *Proc. Third Intern. palynol. Conf.*, Novosibirsk, USSR (in the press).
- ¹⁵ Brooks, J., and Shaw, G., *Chem. Geol.*, **10**, 69 (1972).
- ¹⁶ Potonie, R., and Rehneit, K., in *Sporopollenin* (edit. by Brooks, J., et al.), 295 (Academic Press, 1971).
- ¹⁷ Oehler, D. Z., Schopf, J. W., and Kvenvolden, K., *Science, N.Y.*, **1246** (1972).
- ¹⁸ Silverman, S. R., in *Isotopic and Cosmic Chemistry* (edit. by Craig, H.), 92 (North Holland, Amsterdam, 1964).

¹⁹ Viljoen, M. J., and Viljoen, R. P., in *Upper Mantle Project (Geol. Surv. S. Afr., Spec. Publ. 15, 1969)*.

²⁰ Brock, T. D., and Darland, G. K., *Science, N.Y.*, **169**, 1316 (1970).

²¹ Walter, M. R., *Econ. Geol.*, **67**, 965 (1972).

²² Walter, M. R., Bauld, J., and Brock, T. D., *Science, N.Y.*, **178**, 402 (1972).

²³ Hurley, P. M., Pinson, W. H., Nagy, B., and Teska, T. M., *Earth planet. Sci. Lett.*, **14**, 360 (1972).

Auroral Audibility

INCIDENTAL reference was made to the "eerie electric crackle" of auroras recently¹ by a writer from Unst, the most northerly island of Shetland. A correspondent² who had spent several winters in the Arctic and Antarctic dismissed this crackle as "an auricular illusion well known to polar explorers". But others³⁻⁷ reported that sound (described as "crackling" or "swishing") is indeed associated with auroras. This is widely believed by people living in regions where auroras are common⁸.

The question of occasional auroral audibility is conjectural. There is the strong negative evidence of the numerous scientifically trained observers who have heard nothing even during intense displays. But Chapman⁹ and Beals¹⁰ have summarized positive evidence which cannot lightly be disregarded, especially as entirely independent descriptions form a coherent pattern. According to Beals there are two distinct types of sound: "crackling" and "swishing".

One of the most striking accounts is by the astronomer Jelstrup¹¹, who with his brother "noticed a very curious faint whistling sound distinctly undulatory which seemed to follow exactly the vibrations of the aurora". Störmer's chief assistant, Tjønn¹², has witnessed an aurora which was seemingly accompanied for about 10 min by a noise like "burning grass and spray", the level of the noise rising and falling with the intensity of the light emission. A simultaneous change in the acoustic and luminous intensity (if significant) indicates the source of the sound to be close to the observer¹³.

Harang¹⁴ concluded that because of the strength of the negative evidence many of the statements regarding sounds must be ascribed to "psychological errors" but that some of the statements are so reliable and objective that they must be accepted as relating to a physical effect. In his last comments on the issue Chapman¹⁵ was non-committal.

The suggestion by Amundsen that "What one hears is one's own breath which freezes in the cold air" was taken up by Sverdrup¹⁶ and has since been widely quoted. Sverdrup confirmed that on listening intently a weak "swishing" (which he attributed to the ice crystals formed colliding when falling to the ground) may indeed be heard when the air is at a temperature below -40°C and is calm. In these conditions the sky is generally clear and, in the maximum auroral zones, an aurora is generally visible. Sverdrup argued that, "An observer standing still and watching a brilliant display then hears a weak swishing sound, rising rhythmically, and naturally he assumes that this sound is emitted by the aurora". The "freezing breath" hypothesis was independently put to Beals¹⁰ by F. J. Davies, physicist of the Byrd Antarctic Expedition. Beals was unconvinced, partly because of evidence of auroral noise even when the weather was not extremely cold. It has been suggested that a source of confusion in some places could be the sound of surf breaking on a distant seashore.

Beals¹⁰ sought an explanation in terms of brush discharges from projecting objects. The idea had been advanced earlier¹⁷. Many people have seen brush discharges during auroral displays^{10,18}. The coincidences of the events may have been merely fortuitous. Changes in the atmospheric potential gradient and auroras are certainly not associated, but auroras are accompanied by magnetic storms. In consequence there can be discharges from telephone and other wires. Harang¹⁴ has proposed that these discharges may be the source of at least some auroral noise.

Simply on account of the great heights involved (≥ 70 km) the possibility of sound coming directly from an aurora was immediately dismissed by Chapman⁹ (except that at the time he tended to believe in the rare occurrence of very low auroras) and by Störmer¹². The possibility was not examined even semi-quantitatively, perhaps because an aurora was not thought a likely source of strong acoustic waves. An interesting change of outlook has taken place.

In 1963 Buneman¹⁹ suggested that the undulatory disturbances indicated by radar reflexions from auroras might be plasma acoustic waves excited by the streaming electrons and that these waves couple to ordinary acoustic waves in the dominant neutral background. He and Farley²⁰ treated the phenomenon mathematically. In a footnote Buneman remarked: "Claims that auroras are audible to the human ear reached the author after he developed the theory". This raises the question of whether the acoustic waves generated in an aurora could be perceptible to an observer on the ground. I show that they cannot.

Acoustic waves suffer attenuation due to viscosity and thermal conduction. In passing a distance dz through still air the power flux P of a plane acoustic wave changes by an amount

$$dP = -KPdz \quad (1)$$

where

$$K = A\rho^{-1} \quad (2)$$

with

$$A = 2\pi^2\mu\nu^2/3\nu^3\{(23\gamma - 15)/\gamma\} \quad (3)$$

ρ , μ and γ being the density, viscosity and ratio of the specific heats of the air and ν and v being the frequency and velocity of the wave²¹. I consider a plane wave originating from a level S high above ground level and propagated downwards. For present purposes it is a sufficient approximation to take the atmosphere near S to be isothermal. With ρ_s and H_s the density and scale height at S and P_s the initial power flux, P_G , the power flux reaching the ground is given by

$$P_G = P_s \exp[-AH_s/\rho_s] \quad (4)$$

Numerical substitution gives that at 230 K, which is about the relevant temperature

$$AH_s/\rho_s = 2.3 \times 10^{-7} \{(\rho_0/\rho_s)\nu^2\} \quad (5)$$

in which ρ_0 is the density of air at STP.

An extreme upper limit to P_s in any frequency band is the total power flux of the incident particles producing the aurora. For a great aurora of international brightness coefficient IV this is about 3 W m^{-2} (see ref. 22). To favour the hypothesis under investigation further, I assume that level S is as low as 70 km. In the subarctic (winter warm) model atmosphere of Cole *et al.*²³, ρ_s is then about $8 \times 10^{-2} \text{ g m}^{-3}$.

The absolute values of P_G are less revealing than the values relative to the known²⁴ power flux at the threshold of hearing. Though the ear is most sensitive near 2,000 Hz, where the threshold of hearing is at only about $10^{-5} \mu\text{W m}^{-2}$, the optimum frequency in the present context is at the grave end of the audible frequency range. The calculated upper limit to P_G is +2 bel at 25 Hz (which is just above this end), is +1 bel at 50 Hz, and (now falling very sharply with increase in frequency) is -9 bel at 100 Hz. It is clear from these figures that acoustic waves generated in auroras could not be responsible for the sounds which have been reported.

Infrasound waves from auroras, with pressure amplitudes of a few tenths of 1 N m^{-2} , have been recorded^{13,25}. Because of their long periods, usually between 10 s and 100 s, they pass readily through the atmosphere. The associated power flux is a minute fraction of the total power flux of the particles producing the auroras. There is evidence that arcs moving with supersonic speeds are involved^{26,27}.

No means by which the small pressure changes of the infrasonic waves could produce noise has been proposed.

D. R. BATES

*Department of Applied Mathematics and Theoretical Physics,
Queen's University Belfast,
Belfast BT7 1NN*

Received March 26; revised June 22, 1973.

- ¹ Jones, T., *The Times*, March 5 (1973).
- ² Riley, Q., *The Times*, March 8 (1973).
- ³ Johnston, A., *The Times*, March 22 (1973).
- ⁴ Kaye, C. W., *The Times*, April 5 (1973).
- ⁵ Brown, P. L., *The Times*, April 11 (1973).
- ⁶ Thomas, E., *The Times*, April 16 (1973).
- ⁷ Lea, H., *The Times*, April 19 (1973).
- ⁸ Elvey, C. T., *Adv. Electronics Electron Phys.*, **9**, 1 (1957).
- ⁹ Chapman, S., *Nature*, **128**, 457 (1931).
- ¹⁰ Beals, C. S., *Q. Jl R. met. Soc.*, **59**, 71 (1933).
- ¹¹ Jelstrup, H. S., *Nature*, **119**, 45 (1927).
- ¹² Tjønn, T., cited by Störmer, C., in *Nature*, **141**, 955 (1938) and in *The Polar Aurora* (Clarendon Press, Oxford, 1955).
- ¹³ Campbell, W. H., and Young, J. M., *J. geophys. Res.*, **68**, 5909 (1963).
- ¹⁴ Harang, L., *The Aurorae* (Chapman and Hall, London, 1951).
- ¹⁵ Chapman, S., in *Aurora and Airglow* (edit. by McCormac, B. M.) (Reinhold, New York, 1967).
- ¹⁶ Sverdrup, H. E., *Nature*, **128**, 457 (1931).
- ¹⁷ Störmer, C., *Nature*, **119**, 46 (1927).
- ¹⁸ Simpson, G. C., *Q. Jl R. met. Soc.*, **59**, 185 (1933).
- ¹⁹ Buneman, O., *Phys. Rev. Lett.*, **10**, 285 (1963).
- ²⁰ Farley, D. T., *J. geophys. Res.*, **68**, 6083 (1963).
- ²¹ Lord Rayleigh, *Theory of Sound* (second ed.) (Macmillan, London, 1894).
- ²² Dalgarno, A., in *Auroral Phenomena* (edit. by Walt, M.) (Stanford University Press, 1965).
- ²³ Cole, A. E., Court, A., and Kantor, A. J., in *Handbook of Geophysics and Space Environments* (edit. by Valley, S. L.) (McGraw-Hill, New York, 1966).
- ²⁴ Stephens, R. W. B., and Bate, A. E., *Acoustics and Vibrational Physics* (second ed.) (Edward Arnold, London, 1966).
- ²⁵ Chrzanowski, P., Greene, G., Lemmon, K. T., and Young, J. M., *J. geophys. Res.*, **66**, 3727 (1961).
- ²⁶ Wilson, C. R., and Nichparenko, S., *Nature*, **214**, 1299 (1967).
- ²⁷ Wilson, C. R., *Nature*, **216**, 131 (1967).

Tunnelling Measurements of Vibrational Spectra of Amino Acids and Related Compounds

OBSERVATIONS of inelastic electron tunnelling in doped tunnel junctions have been used to study molecular vibrational spectra of organic molecules including amino acids, fatty acids and related compounds. The potential of the technique as a spectroscopic tool in the analysis of trace amounts of chemical compounds has been explored through a systematic study of the spectra observed for a group of several dozen dopant chemicals. Previous observations of inelastic tunnelling spectra have been limited to a few simple organic acids and alcohols with high vapour pressures^{1,2}. In the experiments described here a system has been developed which allows the preparation of doped tunnel junctions using any reasonably stable chemical compound without danger of cross-contamination of the junction fabrication apparatus and permits the use of a wide range of successive dopants.

The tunnel junctions consist of two thin-film metal electrodes separated by an insulating barrier with the adsorbed molecular species at the barrier-electrode interface. In our experiments Al and Pb electrodes were used, with the insulating barrier formed by oxidation of the Al using the glow discharge technique to form an aluminum oxide layer approximately 20 Å thick. Deposition of both electrodes and growth of the oxide layer was carried out in a conventional bell jar evaporator system. This system was connected by a gate valve to a cylindrical doping system equipped with a removable in-line glass-bellows liquid nitrogen trap which served the dual purpose of

cooling the substrate prior to doping and of preventing contamination of the bell jar evaporator system with dopant chemicals. The systems were evacuated to $\sim 10^{-6}$ torr by separate oil diffusion pumps operated with liquid nitrogen traps. Volatile organic impurities were introduced into the doping chamber from a pre-evacuated glass tube connected to the doping chamber through a glass stopcock. Amino acids and other relatively low vapour pressure organic compounds were placed in a resistively heated tantalum boat located in the doping chamber just below the cooled substrate block. After pumpdown and preparation of the Al electrode and Al_2O_3 barrier the substrate was transferred to the doping chamber and the dopant was evaporated onto the barrier layer by heating the tantalum boat using the appropriate time and temperature. For compounds such as amino acids extremely precise control of temperature was required in order to obtain the correct doping level. Junctions were transferred between the two systems by a rolling carriage mechanism which allowed the complete junction fabrication and doping to be carried out without breaking vacuum. This is absolutely necessary in order to obtain reliable and reproducible inelastic tunnelling spectra. Measurements of tunnelling spectra were performed using second harmonic methods employing a phase-sensitive lock-in amplifier and a high resolution bridge circuit described elsewhere³. The measurements reported here were carried out with the junction immersed in a bath of liquid helium at 4.2 K.

The spectra observed are due to the excitation of either molecular impurity vibrations or modes in the barrier or electrodes^{4,5}. The measurement technique used here consists of recording the second derivative of tunnel current with respect to applied bias voltage as a function of applied bias voltage. At a voltage corresponding to the energy of an inelastic event a new channel for tunnel current opens giving a discontinuity in the conductance (dI/dV) which appears as a peak in the second derivative characteristic. The molecular vibrational spectrum obtained by sweeping the bias voltage over an appropriate range is analogous to a conventional infrared spectrum, although not identical in all respects. An undoped junction (clean junction) shows prominent features which can be identified with electrode or barrier modes and a spectrum obtained from a control junction carried through the complete preparation process without doping is shown in the lowest curve of Fig. 1.

The most prominent feature is the alumina hydrate OH bending mode at 920 cm^{-1} due to trace amounts of water vapour in the system that react with the aluminium oxide layer. Also present are the Al phonon peak at 320 cm^{-1} , the second harmonic of the hydrate OH bend at $1,840\text{ cm}^{-1}$, and the free OH stretch at $3,630\text{ cm}^{-1}$. A CH stretch mode is also observed in the clean junction spectrum. This mode should be absent in a clean junction and probably arises from slight contamination of the junction by pump oil.

Typical spectra from doped junctions are shown in Figs 1 and 2. All of the doped junctions exhibit a very strong CH stretch mode due to the presence of this group in the dopant molecule. A CH bending mode near $1,400\text{ cm}^{-1}$ is also observed; this mode is possibly split into two peaks in the case of the alcohols (for example glycerol and ethylene glycol shown in Fig. 1). Several spectra show a CO-OH peak at $1,610\text{ cm}^{-1}$; a peak at this wave number is notably absent when a carboxyl group is not present as in glycerol and ethylene glycol. In the case of acetamide and trichloroethylene the peak at this wave number is assigned to a CO-NH₂ and a C=C vibration respectively. Another mode common to most doped junctions is the CH rock in the range $400\text{--}800\text{ cm}^{-1}$. In addition a number of spectra show evidence of an OH bending mode at about $1,300\text{ cm}^{-1}$. In the amino acid spectra additional modes of particular interest are the CH₂-OH peak at $1,100\text{ cm}^{-1}$ in serine, the CH-NH-CH peak at $1,210\text{ cm}^{-1}$ in proline, and the possible amine peak at $3,310\text{ cm}^{-1}$ in cysteine. A peak occurs in some spectra at about $1,080\text{ cm}^{-1}$ which is unidentifiable at present but may arise due to an interaction of the molecule

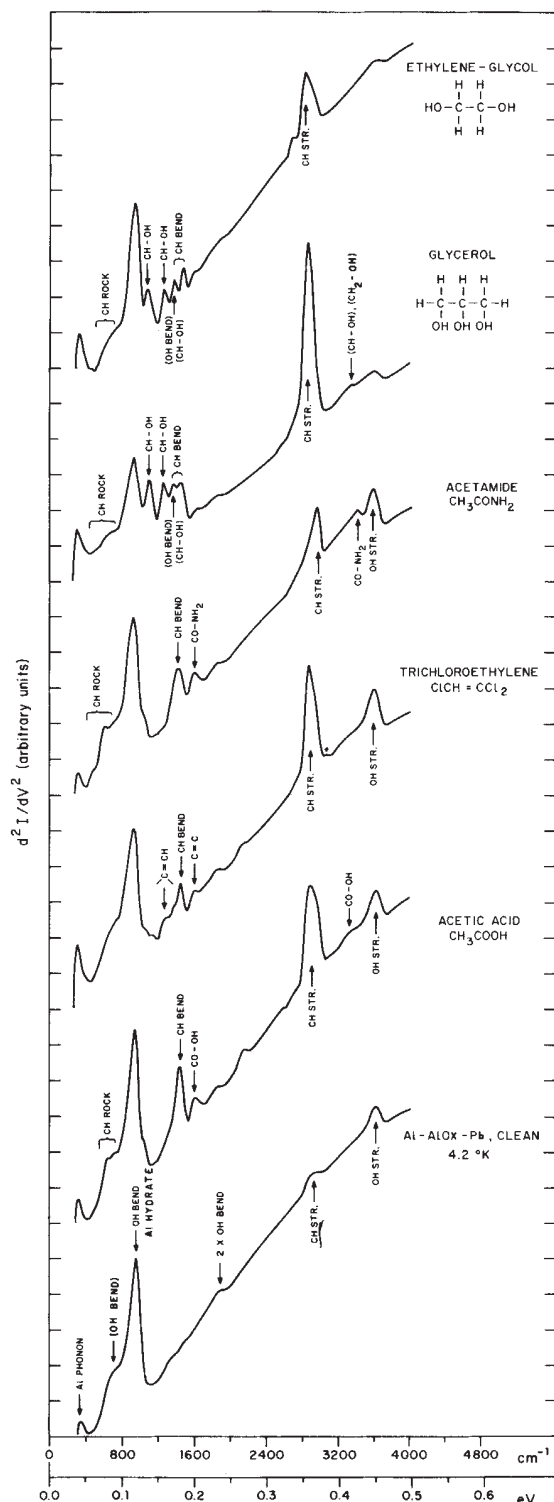


Fig. 1 Typical tunnelling spectra for a variety of organic compounds which are identified to the right of each curve. Peaks in the second derivative (d^2I/dV^2) are identified with specific vibrational modes as indicated by arrows and labels on the graphs. Labels with parentheses indicate alternative identifications in a few cases.

with the Al_2O_3 barrier or the Pb electrode. Another peak occurs intermittently at $3,030\text{ cm}^{-1}$ which may be due to SiH contamination from pump oil.

Our results demonstrate that the inelastic tunnelling technique can be applied to a very wide variety of chemical compounds. The spectra show easily discernible differences between various compounds and clearly demonstrate the potential of the method as an analytical tool for very small quantities of material (less

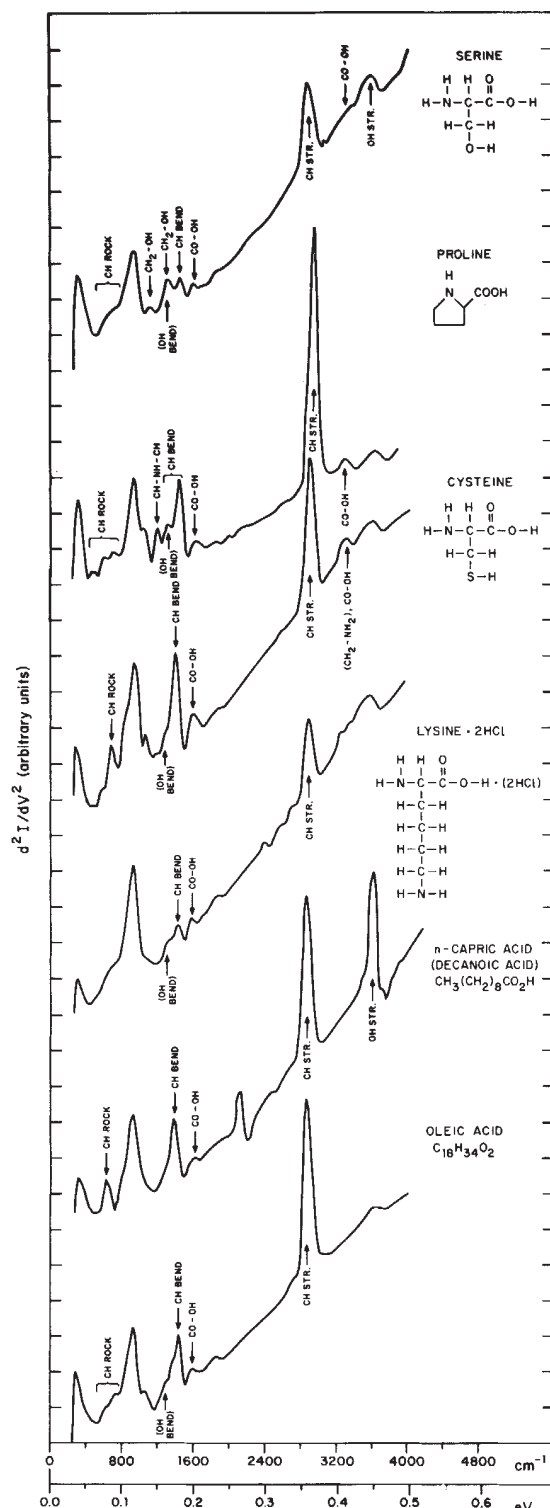


Fig. 2 Tunnelling spectra obtained for representative amino acids and fatty acids. Specific vibrational modes are identified with arrows and labels. When an alternative identification is possible it is indicated in parentheses.

than a monolayer on an area of 0.1–1.0 mm²). Experiments are now in progress to obtain spectra from more complex organic compounds (for example polypeptides) as well as to study the effect of specific molecular structure on the modes already observed.

We thank Drs J. E. Coleman and R. C. Morris for discussions and W. L. Sands for designing and constructing the special glass-bellows liquid nitrogen trap. The work has been supported by the US Atomic Energy Commission and a joint National

Science Foundation grant between the University of Virginia and Yale University.

MICHAEL G. SIMONSEN
R. V. COLEMAN

Physics Department,
University of Virginia,
Charlottesville, Virginia 22901

Received January 22, 1973.

- ¹ Lambe, J., and Jaklevic, R. C., *Phys. Rev.*, **165**, 821 (1968).
- ² Geiger, A. L., Chandrasekar, B. S., and Adler, J. G., *Phys. Rev.*, **188**, 1130 (1969).
- ³ Adler, J. G., and Jackson, J. E., *Rev. sci. Instr.*, **37**, 1049 (1966).
- ⁴ Duke, C. B., *Tunneling in Solids, Solid State Physics Suppl.* (edit. by Seitz, F., Turnbull, D., and Ehrenreich, H.), **10** (Academic Press, New York, 1969).
- ⁵ *Tunneling Phenomena in Solids* (edit. by Burstein, E., and Lundquist, S.), 233 (Plenum, New York, 1969).

BIOLOGICAL SCIENCES

Effects of Cycloheximide on RNA Polymerase Specific Activity and Encystment in *Acanthamoeba castellanii*

TROPHOZOITES of the protozoan *Acanthamoeba castellanii* (Neff) possess at least two distinct RNA polymerase activities which can be separated by DEAE-'Sephadex' A-50 chromatography¹. These enzymes show optimum activities at different magnesium, manganese, and salt concentrations. Polymerase fraction I is also inhibited by cycloheximide, while polymerase fraction II is affected by rifampin and α -amanitin.

When the trophozoites are transferred from an enriched to a minimal salts medium, they differentiate to form a relatively inactive cyst within 24 h. We have demonstrated, however, that for 12 to 15 h after induction of encystment, total RNA polymerase specific activity increases and there is significant uptake of ³H-uridine into both acid-soluble and acid-insoluble cell fractions¹. After 15 h, enzymatic specific activity decreases 60–70% and there is a marked loss of radioactivity both from acid-soluble and acid-insoluble components.

We have now investigated the effects of cycloheximide on the increase in total RNA polymerase specific activity and on the uptake of ³H-uridine by encysting amoebas, and also have examined the effect of cycloheximide on the formation of the end product of differentiation in these organisms (the mature cysts).

Cultures of *A. castellanii* were grown and induced to encyst as previously described^{2,3}. Trophozoites begin to round up and lose the ability to form pseudopodia 5 to 7 h after induction. This rounded cell, the immature cyst, is maximal at 10 to 12 h when it comprises 60% of the population. The population contains 80% mature cysts, characterized by a thick cellulose wall, by 24 h.

The pattern of total RNA polymerase specific activity as a function of encystment in the presence of cycloheximide is illustrated in Fig. 1. The level of inhibitor chosen (500 μ g ml⁻¹) is that which, when added exogenously to medium containing either dividing or encysting *A. castellanii*, completely inhibits cytoplasmic protein synthesis within 30 min as shown by following the incorporation of ³H-leucine into trichloroacetic acid-precipitable material. The drug does not cause cell death for at least 48 h, nor does it prevent the amoebas from dividing or encysting normally once they are removed from the inhibitor and inoculated into fresh medium. Cycloheximide was always added to encystment medium as a sterile-filtered solution.

In the absence of cycloheximide (Fig. 1), there is an approximate doubling in total specific activity of polymerase from 8 to

12 h, that is, when the level of immature cysts is maximal. When the inhibitor is added to cultures of encysting cells, there is no increase in enzyme specific activity at 10 h. Attempts to partially purify 10 h cyst RNA polymerase to the same extent at trophozoite enzyme were unsuccessful¹, so that we could not examine the effect that addition of cycloheximide to encystment medium has specifically on fractions I and II.

As the presence of cycloheximide in the medium prevents the increase in polymerase activity in encysting cells, it was also of interest to examine the effect of the drug on uptake of ³H-uridine into trichloroacetic acid-precipitable material. When encysting cultures are continuously exposed to ³H-uridine, there is significant uptake of the isotope into acid-insoluble material from 0–15 h¹. The results indicated a continued synthesis of RNA during encystment even though there is a net RNA loss per cell. When cycloheximide is included in the encystment medium during ³H-uridine incorporation, however (Fig. 2), there is a 67% inhibition in the uptake of labelled precursor into acid-insoluble material by 4 h and a 79% inhibition by 12 h. There is little incorporation of tritium after 4 h.

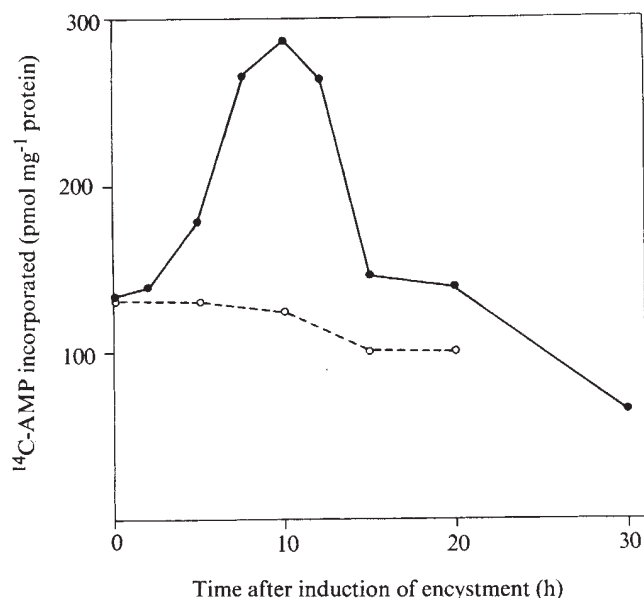


Fig. 1 The effects of cycloheximide on total RNA polymerase specific activity during encystment of *A. castellanii*. Cells were incubated in the presence or absence of 500 $\mu\text{g ml}^{-1}$ cycloheximide (Sigma Chemical Co.) during encystment. At the times indicated RNA polymerase was partially purified through ammonium sulphate fractionation, desalted by chromatography on 'Sephadex' G-25, and assayed as described by Rudick and Weisman¹. Incubations were for 10 min at 37° C. ●, Activity in the absence of cycloheximide; ○, activity in the presence of cycloheximide.

To test the effect of cycloheximide on the ability of *A. castellanii* to differentiate and form mature cysts, the inhibitor was added to encysting cells at various times after induction. The cultures were then allowed to encyst for 24 h before being counted in a haemocytometer. Cells were categorized as trophozoites, immature cysts, or mature cysts.

In the absence of cycloheximide, the number of mature cysts is always high (80–85%) at the end of 24 h and that of immature cysts is low (Fig. 3a). In contrast, when the inhibitor is added to the cultures at any time up to approximately 12 h, immature cysts make up the majority of the population (80–97%), while few mature cysts are visible (Fig. 3b). Only when the drug is administered after 16 h do the mature cysts increase significantly.

The data suggest that cycloheximide added exogenously to

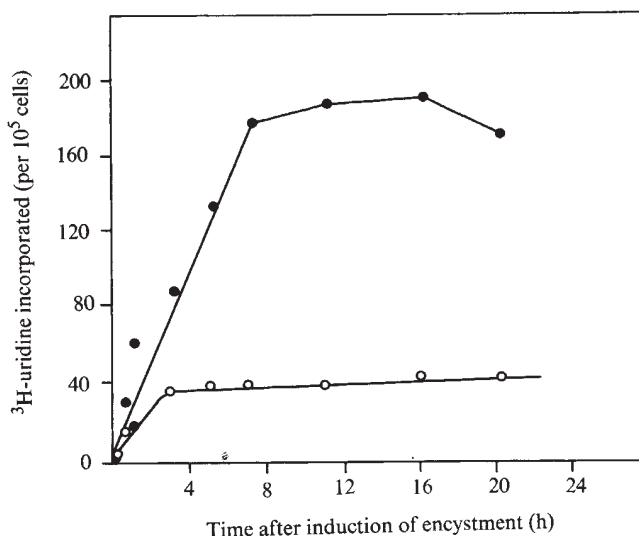


Fig. 2 ³H-Uridine incorporation into trichloroacetic acid-insoluble material with (○) or without (●) 500 $\mu\text{g ml}^{-1}$ cycloheximide. ³H-5'-uridine (25.7 Ci mmol⁻¹; New England Nuclear) was present continuously from time zero at 5 $\mu\text{Ci ml}^{-1}$. Unlabelled uridine was also added at a final concentration of 10⁻⁵ M. Incorporation was stopped by the addition of an equal amount of cold 10% trichloroacetic acid to the culture. Precipitates were collected after 30 min on 0.45 μm 'Millipore' filters and washed several times with 20 ml aliquots of 5% trichloroacetic acid. Filters were dried, placed in a toluene-based scintillation fluid containing 4.9 g l⁻¹ 2,5-diphenyloxazole [PPO] and 0.1 g l⁻¹ 1,4-bis-2 [4-methyl 5-phenyloxazole] benzene, and counted in a Tri-Carb liquid scintillation spectrometer (Packard Instruments Co.).

encysting cells may affect differentiation in at least two possible ways. First, the evidence that cycloheximide inhibits cytoplasmic protein synthesis in general by *A. castellanii* and also prevents the rise in total RNA polymerase specific activity observed at 8 to 12 h may indicate that new protein synthesis is required for differentiation. Thus, the rise in polymerase specific activity may represent new enzyme formation and/or synthesis of protein(s) which modifies polymerase activity. Second, as the presence of cycloheximide in the polymerase assay mixture inhibits

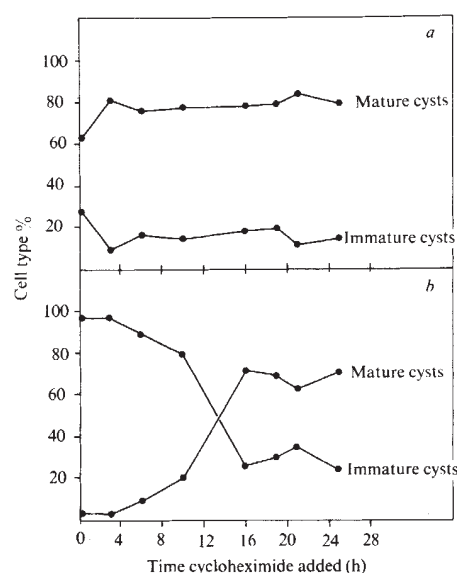


Fig. 3 The effect of cycloheximide on the ability of *A. castellanii* to encyst. The inhibitor (500 $\mu\text{g ml}^{-1}$) was added at various times after induction, then the cells were allowed to encyst for a further 24 h before being counted on a haemocytometer. a, Control cultures, no cycloheximide; b, cycloheximide added at the indicated times.

the trophozoite DEAE-fraction I¹, we may speculate that the increase in total polymerase specific activity at 8 to 12 h represents primarily a rise in fraction I. Of course, due to the instability of the cyst enzyme, we are unable to identify positively which of the activities is enhanced. It is of interest to note that similar RNA polymerases have been purified from vegetative thalli and zoospores of the water mould *Blastocladiella emersonii*^{4,5}, and that in this organism cycloheximide has been reported to inhibit the fraction I enzyme obtained following DEAE-cellulose chromatography.

There have been several reports suggesting that cycloheximide reduces RNA synthesis⁶⁻⁹, and our data from ³H-uridine incorporation studies indicate that the drug at least prevents the uptake of RNA precursor into macromolecules. Attempts to look at pulse-labelled RNA synthesized during encystment by polyacrylamide gel electrophoresis were not successful, as the cells are impermeable to RNA precursors so that it was not possible to obtain RNA with a specific activity high enough to detect reliable peaks of radioactivity in the gels. The gross electrophoretic patterns of RNA isolated from vegetative or encysting cells were similar. Cycloheximide inhibition specifically affects synthesis and processing of ribosomal precursor RNA^{6,7}, probably by acting indirectly through cessation of protein biosynthesis^{7,9}. If *A. castellanii* fraction I enzyme is nucleolar like that of other eukaryotes¹⁰, then the uptake of uridine which we observed may be involved in rRNA synthesis and the presence of cycloheximide may be preventing uptake indirectly by inhibiting fraction I RNA polymerase.

Our data show that cycloheximide blocks the transition from the immature to the mature cyst when added up to about 12 h after the induction of encystment. As the morphological change from the trophozoite to the immature cyst does take place, events occurring in the first 8 h which culminate in the appearance of the immature cyst may not require either new proteins or additional RNA polymerase activity. These results probably do not simply reflect a change in permeability early in encystment, as cycloheximide does stop the incorporation of ³H-leucine into trichloroacetic acid-precipitable material within 30 min of addition. Inhibitor added to the cells after 12 h, however, does not affect morphogenesis. These data indicate that there appears to be a narrow time span from 12 to 14 h after induction when events obligatory for subsequent development must occur. As this time span coincides with the rise in RNA polymerase specific activity, we may speculate that this increase is required before genes for cyst-specific RNA can be transcribed and cyst-specific proteins produced for the transition from immature to mature cysts.

This work was supported by a grant from the Public Health Service (Allergy and Infectious Diseases).

V. L. RUDICK
R. A. WEISMAN

Department of Biochemistry,
The University of Texas Health Science Center,
San Antonio, Texas 78284

Received February 19; revised May 3, 1973.

- ¹ Rudick, V. L., and Weisman, R. A., *Biochim. Biophys. Acta*, **299**, 91 (1973).
- ² Potter, J. L., and Weisman, R. A., *Develop. Biol.*, **28**, 472 (1972).
- ³ Neff, R. J., Ray, S. A., Benton, W. F., and Wilborn, M., *Methods in Cell Physiology*, **1** (edit. by Prescott, D. M.), 55 (Academic Press, 1964).
- ⁴ Horgen, P. A., and Griffin, D. H., *Proc. US Nat. Acad. Sci.*, **68**, 338 (1971).
- ⁵ Horgen, P. A., *J. Bact.*, **106**, 281 (1971).
- ⁶ Willems, M., Penman, M., and Penman, S., *J. Cell Biol.*, **41**, 177 (1969).
- ⁷ Muramatsu, M., Shimada, N., and Higashinakagawa, T., *J. Mol. Biol.*, **53**, 91 (1970).
- ⁸ Wanka, F., and Schrauwen, P. J. A., *Biochim. Biophys. Acta*, **254**, 237 (1971).
- ⁹ Higashinakagawa, T., and Muramatsu, M., *Biochem. Biophys. Res. Commun.*, **47**, 1 (1972).
- ¹⁰ Roeder, R. G., and Rutter, W. J., *Proc. US Nat. Acad. Sci.*, **65**, 675 (1970).

Rapid and Selective Inhibition of the Synthesis of High Molecular Weight RNA in Yeast by Lomofungin

LOMOFUNGIN (1-carbomethoxy-5-formyl-4,6,8-trihydroxyphenazine), an antibiotic from *Streptomyces lomodensis*, inhibits the growth of bacteria, yeasts and fungi^{1,2}. It has been suggested that in *Saccharomyces cerevisiae*, the primary action of the antibiotic is an inhibition of RNA synthesis^{2,3}. Cano, Kuo and Lampen⁴ found that lomofungin inhibits three DNA-dependent RNA-polymerases extracted from *S. cerevisiae*. We have characterized the effects of lomofungin on RNA synthesis in the fission yeast, *Schizosaccharomyces pombe*.

S. pombe strain 132 was grown in a minimal medium, EMM 2 (ref. 5). Cells from exponential phase cultures were collected by centrifugation and resuspended in medium containing lomofungin. Control cells were resuspended in medium alone. Measurements of RNA synthesis were made during various periods after addition of lomofungin by supplying ³²P-phosphate, ³H-adenine or ³H-uridine.

Synthesis of ribosomal RNA (rRNA) and of polydisperse RNA was markedly inhibited within a few minutes of addition of lomofungin (Fig. 1a, b; Table 1). Pulse-labelling experiments carried out 15–35 min after addition of the antibiotic showed that the synthesis of polydisperse RNA, presumed to contain messenger RNA, had virtually ceased. Synthesis of rRNA was also almost completely inhibited (Fig. 1c; Table 1). Ribosomal and polydisperse RNA syntheses had very similar sensitivities to varying lomofungin concentrations (Table 2).

Table 1 Inhibition of RNA Synthesis after Addition of 60 µg ml⁻¹ Lomofungin

Period of labelling (min)	Label	Percentage inhibition of incorporation into			
		rRNA*	Poly-disperse RNA	5S RNA	tRNA
1–6	³ H-Adenine	57	53	—	—
15–30	³ H-Uridine	87	—	—5	—6
30–35	³ H-Adenine	95	97	6	34

The data were derived from gel scans such as those shown in Figs. 1 and 2. The total counts incorporated into an RNA species were found by adding the radioactivities of the individual gel slices comprising the radioactivity peak of that RNA, allowing for the background of polydisperse radioactivity in the gel. The inhibition of RNA synthesis by lomofungin treatment was found by comparison of incorporation in lomofungin-treated cells with control cells labelled in the same conditions.

* Includes the two mature species of rRNA and the rRNA precursors.

Table 2 Inhibition of RNA Synthesis by Various Concentrations of Lomofungin

Lomofungin (µg ml ⁻¹)	1	10	60
% Inhibition of rRNA*	63	73	94
Polydisperse RNA	62	65	81

Cells were labelled with ³²P-phosphate (Radiochemical Centre, Amersham) 10–30 min after adding lomofungin at various concentrations. Control cells were labelled without lomofungin. The total incorporation into rRNA and polydisperse RNA was measured from gel separations as explained in Table 1, and percentage inhibitions calculated by comparison with the control values.

* Includes the two mature species of rRNA and the rRNA precursors.

In cells pulse-labelled 1–6 min after the addition of lomofungin, the proportion in the precursor rRNA peak of the total radioactivity incorporated into all rRNAs (precursor* plus mature rRNAs) was greater than the proportion in precursor rRNA in similarly labelled control cells (Fig. 1a, b). This suggests that lomofungin not only inhibited the synthesis of

rRNA but also inhibited the processing of rRNA precursors to mature rRNAs.

In direct contrast to its strong inhibition of the synthesis of high molecular weight RNA, lomofungin did not inhibit the synthesis of low molecular weight RNAs. Figure 2 and Table 1 show that the synthesis of the 5S rRNA was completely unimpaired by lomofungin treatment even 30–35 min after addition of the drug. Transfer RNA (tRNA) synthesis was not inhibited 15–30 min after lomofungin addition, and slightly inhibited later (Table 1).

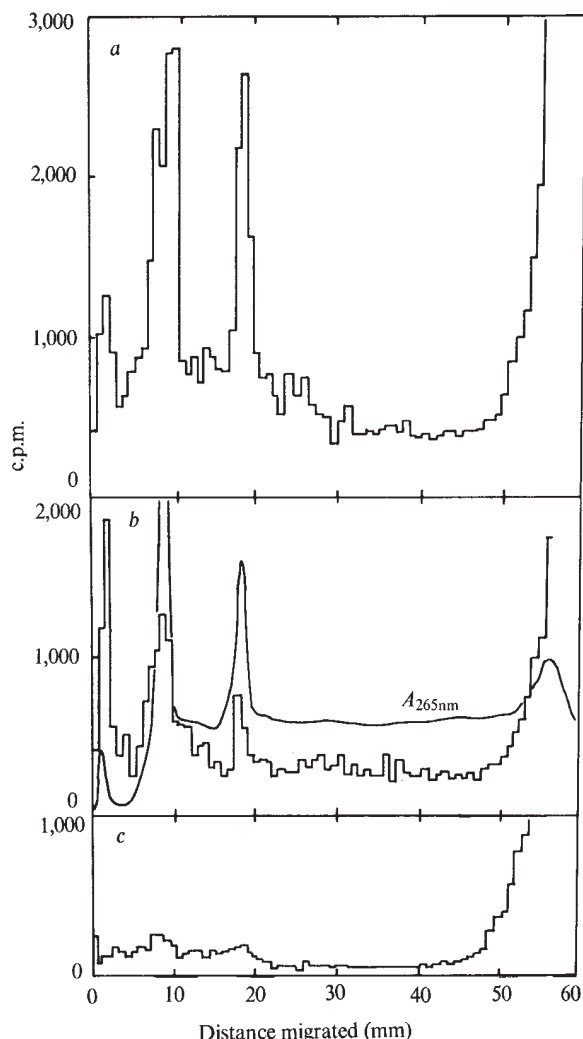


Fig. 1 Polyacrylamide gel electrophoresis of nucleic acids extracted from *S. pombe*. Cells were incubated with ^3H -adenine (specific activity $22.9 \text{ mCi mmol}^{-1}$; Radiochemical Centre, Amersham) at $25 \mu\text{Ci ml}^{-1}$ for 5 min periods. *a*, Control; *b*, 1–6 min after addition of lomofungin ($60 \mu\text{g ml}^{-1}$); *c*, 30–35 min after addition of lomofungin ($60 \mu\text{g ml}^{-1}$). Cells were chilled at the end of the radioactive incubation, washed twice by centrifugation with ice-cold water and suspended in 2% tri-isopropyl-naphthalene sulphonate; 0.1 M Tris-HCl, pH 9.0. The cells were broken in an Eaton press⁵. The homogenate was deproteinized by emulsifying once with 50% v/v chloroform, 50% v/v of the phenol/*m*-cresol mixture of Kirby¹⁸ and twice with the phenol/*m*-cresol mixture. Nucleic acids were precipitated by making the aqueous phase 0.3 M NaCl and adding 2.5 volumes of ethanol. After three cycles of purification by reprecipitation¹⁴, the nucleic acids were fractionated by electrophoresis on 2.6% polyacrylamide gels¹⁵ for 2.5 h at 5 mA per gel, 8 V per cm gel length. Gels were scanned for absorbance at 265 nm in a Joyce-Loebl Gel Scanner, frozen in solid CO_2 and sliced transversely at 0.5 mm intervals with a Mickle Gel Slicer. Radioactivity in each gel slice was determined as described¹⁶. The continuous line shows absorbance at 265 nm; the histograms show radioactivity. The ultraviolet absorbance peaks are: at 10 and 19 mm, 25S and 18S rRNA; at 55 mm, tRNA. The radioactivity peak at 3 mm is the rRNA precursor.

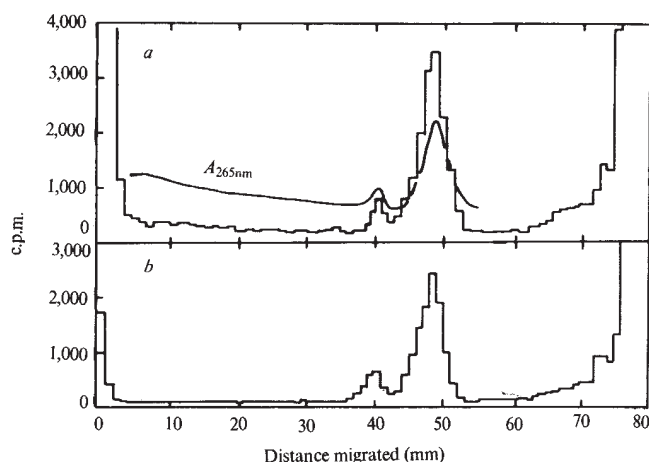


Fig. 2 Polyacrylamide gel electrophoresis of *S. pombe* RNA. Cells were incubated with $25 \mu\text{Ci ml}^{-1}$ ^3H -adenine for 5 min (*a*, control) or 30–35 min after addition of $60 \mu\text{g ml}^{-1}$ lomofungin (*b*). Extraction of RNA and subsequent treatment were as described in Fig. 1. Electrophoresis was on 7.5% acrylamide gels for 2.5 h. The peaks on the ultraviolet absorbance scan (continuous line) are 5S rRNA at 40 mm and tRNA at 49 mm. The radioactivity at 70–80 mm is free ATP.

It is possible that tRNA might have become labelled by turnover of its terminal CCA triplet⁷ rather than by *de novo* synthesis, so masking any effect of lomofungin on tRNA synthesis. Labelling of tRNA by terminal triplet turnover when the isotope supplied was ^3H -uridine could only occur if the cell were actively synthesizing ^3H -CTP through amination of ^3H -uridine. To test this possibility, ^3H -uridine-labelled RNA from cells incubated with and without lomofungin was hydrolysed in 0.5 N NH_4OH ; nucleotides were separated by high voltage electrophoresis⁸ and radioactivity in each was determined. The radioactivity associated with the cytidylic acid ultraviolet absorbance marker spot was about 1% of the radioactivity in the uridylic acid spot. Therefore the possibility that significant labelling of tRNA by terminal triplet turnover occurred when ^3H -uridine was supplied is excluded.

The uninhibited synthesis of 5S and tRNA which occurred in lomofungin-treated cells at the same time as inhibition of rRNA and polydisperse RNA synthesis makes it unlikely that the inhibition of RNA synthesis by lomofungin could be indirect, that is by inhibition of some related aspect of cell metabolism, or by inhibition of the uptake of isotope. We were also able to exclude these explanations by direct experiment. Protein synthesis, measured by the incorporation of ^3H -leucine into trichloroacetic acid-precipitable material, showed a marked impairment only after 30–40 min of treatment by lomofungin at $60 \mu\text{g ml}^{-1}$. The growth of cells, measured by the change in optical density of the culture, continued for 60 min after addition of $60 \mu\text{g ml}^{-1}$ lomofungin. Kuo, Cano and Lampen⁹ found similar effects of lomofungin on protein synthesis and cell growth in *Saccharomyces cerevisiae*. The slow inhibition of protein synthesis and cell growth by lomofungin, compared with the rapid inhibition of RNA synthesis, suggests that lomofungin did not inhibit RNA synthesis indirectly by inhibiting some other aspect of cell metabolism such as energy supply. Lomofungin at $60 \mu\text{g ml}^{-1}$ did inhibit the uptake of ^3H -adenine by *S. pombe*, but only by 40% over the first 30 min after addition of lomofungin. This was much less than the inhibition of incorporation of ^3H -adenine into RNA (Table 1); therefore the measured inhibition of RNA synthesis was not a consequence of inhibited uptake of isotope.

We conclude that lomofungin selectively inhibits the synthesis of rRNA and polydisperse RNA, while having no effect on the synthesis of tRNA or 5S RNA. In comparison, Price and Penman¹⁰ found that the synthesis of 5S and tRNA by isolated HeLa cell nuclei was not inhibited by α -amanitin,

whereas the synthesis of polydisperse RNA was. But α -amanitin does not inhibit rRNA synthesis¹¹.

Unlike actinomycin D (ref. 12), lomofungin enters the yeast cells and inhibits RNA synthesis very rapidly. This antibiotic will prove of value in studies of the mechanisms of control of enzyme synthesis (refs. 2 and 9).

This work was supported by research grants from the MRC and SRC. We thank Drs Cano, Kuo and Lampen for allowing us to see their results before publication, and Dr George B. Whitfield of the Upjohn Company, Kalamazoo, Michigan, USA, for the gift of lomofungin.

R. S. S. FRASER
J. CREANOR
J. M. MITCHISON

Department of Zoology,
University of Edinburgh,
West Mains Road, Edinburgh EH9 3JT

Received March 16, 1973.

- ¹ Johnson, L. E., and Dietz, A., *Appl. Microbiol.*, **17**, 755 (1969).
- ² Lampen, J. O., Kuo, S.-C., and Cano, F., in *Yeast, Mould and Plant Protoplasts* (Academic, NY, 1973).
- ³ Gottlieb, D., and Nicolas, G., *Appl. Microbiol.*, **18**, 35 (1969).
- ⁴ Cano, F., Kuo, S.-C., and Lampen, J. O., *Antimicrob. Ag. Chemother.* (in the press).
- ⁵ Mitchison, J. M., in *Methods in Cell Physiology* (edit. by Prescott, D. M.), **4**, 131 (Academic, NY, 1970).
- ⁶ Grierson, D., Rogers, M. E., Sartirana, M. L., and Loening, U. E., *Cold Spring Harbor Symp. Quant. Biol.*, **35**, 589 (1970).
- ⁷ Daniel, V., and Littauer, U. Z., *J. Mol. Biol.*, **11**, 692 (1965).
- ⁸ Sanger, F., and Brownlee, G. G., in *Methods in Enzymology* (edit. by Grossman, L., and Moldave, K.), **12**, 361 (Academic NY, 1967).
- ⁹ Kuo, S.-C., Cano, F., and Lampen, J. O., *Antimicrob. Ag. Chemother.* (in the press).
- ¹⁰ Price, R., and Penman, S., *J. Mol. Biol.*, **70**, 435 (1972).
- ¹¹ Zylber, E. A., and Penman, S., *Proc. US Nat. Acad. Sci.*, **68**, 2861 (1971).
- ¹² Mitchison, J. M., Creanor, J., and Sartirana, M. L., in *Yeast, Mould and Plant Protoplasts* (Academic, NY, 1973).
- ¹³ Kirby, K. S., *Biochem. J.*, **96**, 266 (1965).
- ¹⁴ Loening, U. E., *Biochem. J.*, **113**, 131 (1969).
- ¹⁵ Loening, U. E., *Biochem. J.*, **102**, 251 (1967).
- ¹⁶ Fraser, R. S. S., and Loening, U. E., *Eur. J. Biochem.*, **34**, 153 (1973).

Influence of Cyclic AMP on Early Events of the Immune Induction

In several recent reports it has been suggested that cell division is regulated by 3,5-cyclic adenosine monophosphate (cyclic AMP)¹. Human diploid fibroblasts² as well as transformed tissue culture cells³ seem to respond with decreased mitotic activity after treatment with cyclic AMP. Studies with human peripheral lymphocytes⁴ indicate that cyclic AMP and cyclic GMP may be involved during cell division. In general the latter studies were performed with no specific mitogenic stimulants to induce cellular proliferation. In this report we present data which indicate that cyclic AMP is able to disturb early events in specific immune induction of mouse lymphocytes *in vitro*. We conclude that these early events may be related to early events of cell division.

Spleen cell cultures were prepared according to the method of Mishell and Dutton⁵. Sheep erythrocytes (SRBC) were used as antigen and added to lymphocytes at the beginning of *in vitro* incubation. They remained in culture until collection at day 4. A dose of 3 to 5 $\times 10^6$ SRBC per 10⁷ spleen cells was used. 3,5-Cyclic adenosine monophosphate (Boehringer, Mannheim) was applied at different times after the addition of antigen.

Mitomycin C (Serva, Heidelberg) was used to inhibit DNA synthesis in lymphocytes which had already been exposed to antigen. We found that a dose of 10 μ l mitomycin C ml⁻¹ cell suspension blocks antibody production *in vitro*,⁶ so we always used this dose in our experiments. Both inhibitors, cyclic AMP and mitomycin C, remained in cultures until the cells were harvested. A possible influence on cell viability was checked by trypan blue dye exclusion tests.

After 4 days of incubation *in vitro* spleen cells were collected and tested for antibody-producing cells with a modified Jerne plaque technique⁷. A culture period of 4 days was found to yield a maximal PFC-response⁸. Spleen cells were treated with four different doses of cyclic AMP in the presence of SRBC (Table 1).

After treatment with cyclic AMP the number of plaque-forming cells (PFC) on day 4 is markedly decreased. When cyclic AMP is present in cultures in a dose of 10⁻³ mol no antibody-forming cells can be detected with the haemolytic plaque assay. Additions of 10⁻⁵ mol and 10⁻⁶ mol cyclic AMP were without significant effect. A dose of 10⁻⁴ mol cyclic AMP gave intermediate values. We used a dose of 10⁻³ mol cyclic AMP as an inhibitory dose in further studies of early events in the immune response.

In these experiments cell cultures were treated with 10⁻³ mol cyclic AMP at different times after the addition of antigen. A significant reduction of the immune response *in vitro* (always tested on day 4) is found when cyclic AMP is applied to lymphocytes not later than 12 h after the antigen (Table 2).

When administered 24 h after antigen, 10⁻³ mol cyclic AMP seems to be unable to alter the number of plaque-forming cells significantly.

In order to inhibit DNA synthesis as well as cellular proliferation, mitomycin C was given to cell cultures in a dose of 10 μ g ml⁻¹ either at the beginning of the immune induction, or 24 h after the addition of the antigen, at the time when cyclic AMP had lost its inhibitory influence. In Table 2 the results of these experiments are summarized. After treatment with mitomycin C antibody production is completely suppressed *in vitro*. There is no difference if mitomycin C is added to culture dishes at the beginning of the immune induction, that is, together with the antigen, or 24 h later.

Clonal proliferation is an essential feature of the immune response. After antigenic stimulation, lymphocytes differentiate into antibody-forming cells as well as non-producing memory cells. This differentiation occurs during cellular proliferation⁹. It has been shown that one critical cycle of DNA replication must occur for lymphocyte differentiation during the induction period of an immune response⁹.

Table 1 Effect of Different Doses of Cyclic AMP on the Immune Response *in vitro*

Experiment	Control without cyclic AMP (PFC/10 ⁶ spleen cells)	10 ⁻³ mol cyclic AMP (PFC/10 ⁶ spleen cells)	10 ⁻⁴ mol cyclic AMP (PFC/10 ⁶ spleen cells)	10 ⁻⁵ mol cyclic AMP (PFC/10 ⁶ spleen cells)	10 ⁻⁶ mol cyclic AMP (PFC/10 ⁶ spleen cells)
I	184	0	93	152	Not done
II	265	0	44	214	209

Each value is the mean of six PFC determinations performed on a pool of six to eight culture dishes.

Table 2 Treatment of Cell Cultures with 10^{-3} mol Cyclic AMP and 10 μ g Mitomycin C/ml Cell Suspension

Experiment	Control without cyclic AMP (PFC/ 10^6 s.c.)	Interval between antigen stimulation and addition of inhibitor				Mitomycin C	
		Cyclic AMP					
		0 h (PFC/ 10^6 s.c.)	6 h (PFC/ 10^6 s.c.)	12 h (PFC/ 10^6 s.c.)	24 h (PFC/ 10^6 s.c.)	0 h (PFC/ 10^6 s.c.)	24 h (PFC/ 10^6 s.c.)
I	164	0	n.d.	n.d.	140	n.d.	n.d.
II	250	0	3	n.d.	215	n.d.	n.d.
III	202	0	0	7	184	0	0
IV	145	0	0	n.d.	53	0	0

Each value is the mean of six PFC determinations performed on a pool of six to eight culture dishes.
n.d.: not done. s.c.: spleen cells.

Our data indicate that exogenous application of cyclic AMP can prevent lymphocytes from differentiation to antibody-producing cells, but only when cyclic AMP is present during a critical period of the immune induction. After this critical time differentiation is not disturbed by cyclic AMP, whereas inhibition of DNA synthesis by mitomycin C still prevents further differentiation. The shorter period of sensitivity towards cyclic AMP, compared with mitomycin C, suggests a different mechanism of action. Mitomycin C is known to interfere with DNA replication; cyclic AMP, however, seems to act at an earlier stage during the immune induction. As it is known that antigenic contact with lymphocytes occurs at specific receptor sites of the lymphocyte membrane, we suggest that after antigenic contact a cell surface alteration might lead to a decrease in the intracellular level of cyclic AMP, which could be a signal for DNA activation, cellular differentiation and proliferation. This decrease might be prevented by exogenous cyclic AMP in these experiments.

Our results differ from those of Braun and co-workers¹⁰. They found enhanced activity of immune cells subjected to cyclic AMP after stimulation with synthetic polynucleotides. This is at variance with the results of other workers^{11,12} on the involvement of cyclic AMP in mitotic processes.

In summary, the addition of either cyclic AMP or mitomycin C leads to inhibition of antibody formation measured as the number of PFC in cultures of spleen cells after four days incubation. In the case of cyclic AMP a reduction of the number of PFC is found only when it is added within 12 h after exposure to antigen; 24 h after antigen exposure, cyclic AMP has no inhibitory effect. Treatment of cell cultures with mitomycin C after 24 h, however, still leads to inhibition of the number of antibody-forming cells. We conclude that exogenous cyclic AMP interferes with early events during activation of immunocompetent cells, preceding DNA replication and clonal proliferation.

This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 138 Biologische Grenzflächen und Spezifität. We thank Professor E. Weiler for helpful discussion and Mrs M. Behrens for technical assistance.

RITA BÖSING-SCHNEIDER
HUBERT KOLB

Immunology Unit, Division of Biology,
University of Konstanz, 775 Konstanz

Received March 9, 1973.

- ¹ Sheppard, J. R., and Prescott, D. M., *Exp. Cell. Res.*, **75**, 293 (1972).
- ² Froehlich, J. E., and Rachmeler, M., *J. Cell Biol.*, **55**, 19 (1972).
- ³ Otten, J., Johnson, G. S., and Pastan, I., *Biochem. Biophys. Res. Commun.*, **44**, 1192 (1971).
- ⁴ Hadden, J. W., Hadden, E. M., Haddox, M. K., and Goldberg, N. D., *Proc. US Nat. Acad. Sci.*, **69**, 3024 (1972).
- ⁵ Mishell, R. J., and Dutton, R. W., *J. Exp. Med.*, **126**, 423 (1967).
- ⁶ Bösing-Schneider, R., and Kindred, B., *Cell. Immunol.*, **5**, 593 (1972).
- ⁷ Bösing-Schneider, R., and Weiler, I. J., *Europ. J. Immunol.*, **1**, 344 (1971).
- ⁸ Cunningham, A. J., *Immunology*, **16**, 621 (1969).

⁹ Feldman, M., *Cell. Immunol.*, **4**, 351 (1972).

¹⁰ Braun, W., Ishizuka, M., Winchurch, R., and Webb, D., *Ann. NY Acad. Sci.*, **185**, 417 (1971).

¹¹ Sheppard, J. R., *Nature New Biology*, **236**, 14 (1972).

¹² Burger, M. M., Bombik, B. M., Breckenridge, M. McL., and Sheppard, J. R., *Nature New Biology*, **239**, 161 (1972).

Long-term Stimulation of Cat Fast-twitch Skeletal Muscle

THE recent demonstration by Sréter *et al.*¹ that long-term stimulation of rabbit fast-twitch muscle induces the production, within the muscle, of myosin light chains characteristic of slow-twitch muscle has encouraged us to publish some of our own preliminary observations on the effects of long-term stimulation in the cat.

Vrbová² and Salmons and Vrbová³ have demonstrated that prolonged stimulation of skeletal muscle can alter the time course of the muscle's isometric twitch response. We have examined the force velocity characteristics of chronically indirectly stimulated muscles.

The left seventh lumbar ventral root was selected and to avoid simultaneous stimulation of sensory nerve fibres we decided to divide the left sixth and seventh lumbar and first sacral dorsal roots. Cats weighing 2.4–3.6 kg were anaesthetized with 'Nembutal' (40 mg kg⁻¹) and at aseptic operation either a left sided L6–S1 intradural deafferentation was performed or deafferentation followed by the implantation of electrodes on the seventh ventral root. In those animals subjected to stimulation, pulses of approximately 0.3 ms duration, 2.6 V intensity and a frequency of 10 s⁻¹ were applied to the electrodes from a subminiature stimulator mounted on a vertebral spinous process. Cats tolerated the procedure extremely well, and the efficiency of the stimulator could be checked by noting the tremulous contraction of the left hindlimb. Up to the time of writing, animals have been examined up to 8 weeks after operation. At the terminal experiment the isometric and isotonic contractions of the medial head of the deep digital flexor (flexor digitorum longus, FDL) were recorded.

In the series of nine control cats unilateral deafferentation alone for 28–50 d produced no statistically significant differences (at the 5% level) between the mean values obtained from the FDL muscles on the two sides for isometric twitch time to peak, twitch tension, tetanic tension, tetanus-twitch ratio or muscle weight.

Figure 1 illustrates our confirmation of the earlier observation of Salmons and Vrbová³. After 41 d of maintained stimulation of the left VRL7 the isometric twitch response of the muscle fibres innervated by that ventral root is greatly slowed compared with the twitch response obtained from the right VRL7 of the same animal. Note also the prolonged half-relaxation time on the stimulated side (Fig. 1b), a characteristic of normal slow-twitch mammalian muscle. In the present series of experiments the mean time to peak of unstimulated (normal and deafferentated) FDL muscles was 22 ms and the time to peak of soleus muscles (normal and deafferentated) 75 ms.

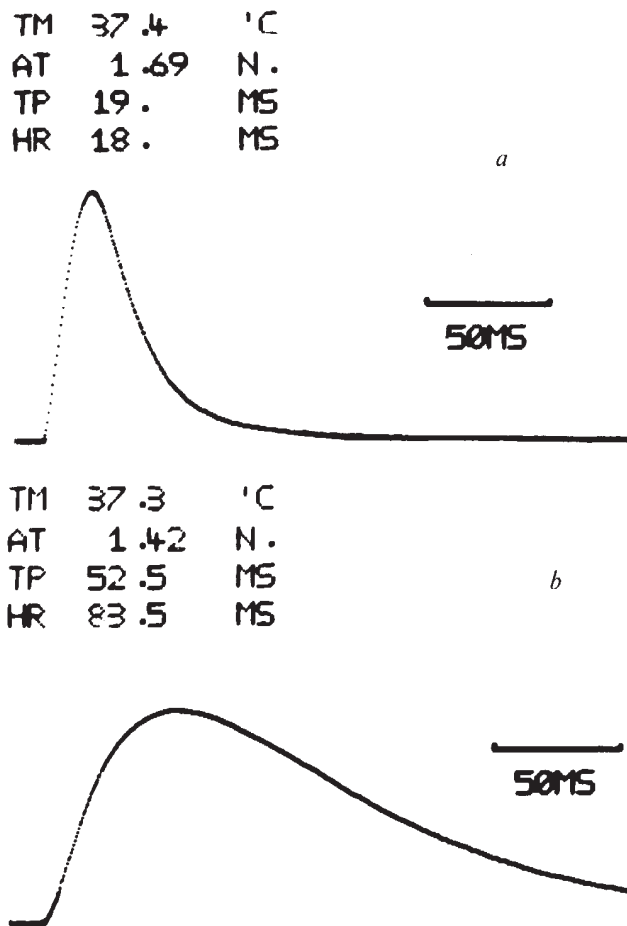


Fig. 1 Twitch responses obtained from the right and left FDL muscles of a cat which had undergone long-term stimulation of the left seventh ventral root (LT VRL7). *a*, Response after maximal stimulation of the right seventh lumbar ventral root (RT VRL7); *b*, response after maximal stimulation of the LT VRL7. *a*: Muscle temperature (TM) = 37.4° C, active tension (AT) = 1.69 N; time to peak (TP) = 19.0 ms, half relaxation time (HR) = 18.0 ms. *b*: TM = 37.3° C, AT = 1.42 N, TP = 52.5 ms, HR = 83.5 ms.

Figures 2 and 3 illustrate the isotonic results obtained from a single stimulated animal compared with those from a deafferented but unstimulated control animal.

The left hand side of Fig. 2 illustrates the force velocity curves obtained by stimulating first the L6 and then the L7 ventral roots of the control animal. The maximum unloaded shortening velocities are similar, being 3.17 and 3.34 muscle lengths s^{-1} respectively. In the present series the values of maximal shortening velocity obtained from unstimulated muscles (whether control or deafferented) were similar to the values found in a large series of normal animals (Buller, Kean and Ranatunga, unpublished results). By contrast the right hand side of Fig. 2 shows that the muscle fibres innervated by the chronically stimulated L7 root have a greatly reduced maximum shortening velocity compared with the muscle fibres innervated by the unstimulated L6 root (1.21 compared with 2.43 muscle lengths s^{-1}). The maximum shortening velocity of the muscle fibres innervated by this L6 root, though slower than the value found in the control animal, still lies within the normal range for FDL muscle. By contrast the muscle fibres innervated by the L7 ventral root produce a force velocity curve similar to that found in normal cat slow-twitch soleus muscle.

Figure 3 illustrates the force velocity curves obtained by stimulating the motor nerves of the same two animals. On the left the curves obtained from the right and left sides of the

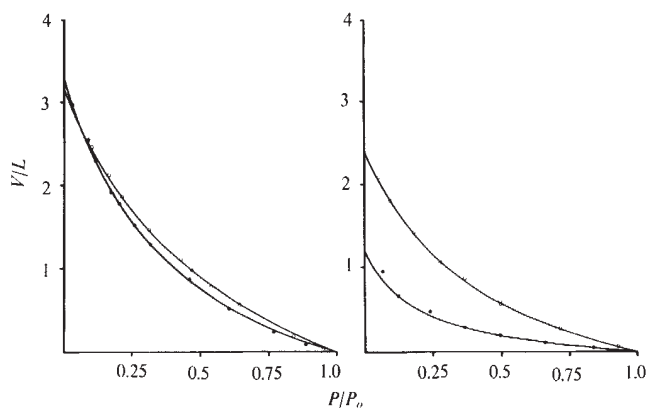


Fig. 2 Force velocity curves obtained by plotting velocity of FDL shortening, measured in muscle lengths s^{-1} (ordinate) against load, expressed as a fraction of the isometric tetanic tension P_0 (abscissa). $\circ$, Results obtained on stimulation of the sixth lumbar ventral root; $\bullet$, Results obtained on stimulation of seventh lumbar ventral root. Left, control animal; right, animal that had received long-term stimulation of the seventh lumbar ventral root. The curves are drawn to Hills equation $(P+a)(V+b) = (P_0+a)b$, the constants a and b being obtained graphically.

control (unstimulated) animal which had undergone a left sided deafferentation 49 d previously superimpose almost exactly. This is characteristic of normal animals in which the side to side variability is found to be far less than the animal to animal variability. On the right of Fig. 3 the force velocity curve obtained on stimulation of the left motor nerve is seen to have a greater curvature than the force velocity relationship obtained from the unstimulated right side. This is because the left FDL contained a mixture of motor units, some fast twitch (innervated by L6) and some resembling slow twitch (innervated by L7), the latter having a more pronounced curvature in their force velocity relationship (see Fig. 2).

These results indicate that prolonged stimulation of cat fast-twitch muscle at a frequency of approximately 10 s^{-1} markedly reduces the muscle's maximum unloaded shortening velocity, and also increases the curvature of the force velocity relationship. Taken with the recent observation of Sréter *et al.*¹ that prolonged stimulation of fast twitch muscle reduces the specific activities of both Ca^{2+} and K^{+} EDTA-activated ATPase of the myosin, these results add further weight to the suggestion by Bárány⁴ that the myosin ATPase activity is the limiting factor determining the maximum shortening velocity of a skeletal muscle.

Although our results clearly confirm and extend the observa-

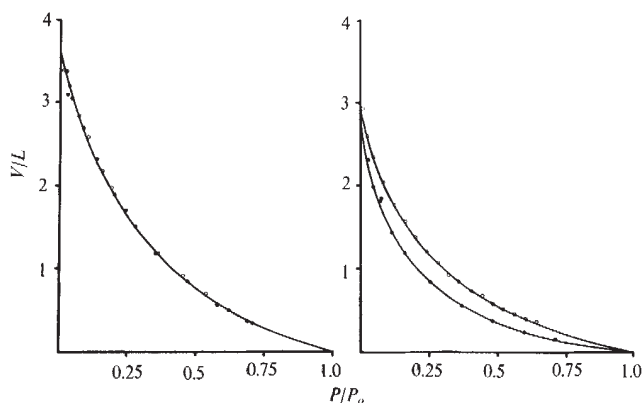


Fig. 3 Force velocity curves, plotted as in Fig. 2, obtained on maximal stimulation of motor nerves to FDL muscles. $\circ$, Results obtained on stimulation of right motor nerves; $\bullet$, results obtained on stimulation of left motor nerves. Left, control animal; right, animal that had received long-term stimulation of the seventh lumbar ventral root.

tions of Salmons and Vrbová³ they do not indicate whether the altered mechanical responses of the muscle follow directly from the increased frequency of motor nerve impulses or indirectly through some such mechanism as an associated increased rate of axonal transport.

W. S. AL-AMOOD
A. J. BULLER
R. POPE

Department of Physiology,
University of Bristol,
The Medical School,
Bristol BS8 1TD

Received March 2, 1973.

¹ Sréter, F. A., Gergely, J., Salmons, S., and Romanul, F., *Nature new Biol.*, **241**, 17 (1973).

² Vrbová, Gerta, *J. Physiol., Lond.*, **185**, 17P (1966).

³ Salmons, S., and Vrbová, Gerta, *J. Physiol., Lond.*, **201**, 535 (1969).

⁴ Bárány, M., *J. gen. Physiol.*, **50**, 197 (1967).

Depression of the T Cell Phenomenon of Contact Sensitivity by T Cells from Unresponsive Mice

Gershon and Kondo¹ provided evidence that T cells from unresponsive mice depressed the antibody response of normal mice to sheep erythrocytes, and introduced the term "infectious tolerance". Jacobson *et al.*² showed that T cells were responsible for the prolonged allotypic suppression in mice which may be a model for immunological

unresponsiveness. Unresponsiveness to picryl chloride (PCI) produced by pretreatment with picryl sulphonic acid (PSA) is also an example of infectious tolerance (positive unresponsiveness)³. In particular, the transfer of lymph node cells from unresponsive to normal mice limited the recipient's contact sensitivity response to PCI. These findings raised the question whether the suppressor cell responsible for positive unresponsiveness was a T cell.

Donor mice were made specifically unresponsive by five injections of 5 mg PSA intravenously twice weekly and left for 10 d³. Other donor mice received one injection and were left 4 d. Anti- θ "serum" was prepared by six weekly injections of CBA thymus cells into AKR mice and ascitic fluid produced by the injection of Sarcoma 37 cells⁴. The serum was inactivated at 56° C and validated *in vitro* by its ability to kill thymus and lymph node cells and its lack of effect on B cell division of mouse spleen cells induced *in vitro* by endotoxin⁵. In some experiments the serum was absorbed with one-fifth of its volume of AKR or CBA brain or thymus⁶. Guinea-pig complement (C) was absorbed with agarose, followed by CBA red cells and a mixture of CBA lymphoid cells⁵.

Lymphoid cells from unresponsive mice (suppressor cells) at 2×10^8 ml.⁻¹ were mixed with an equal volume of one-third anti- θ "serum" and incubated for 30 min at 37° C. They were then washed and incubated with one-third complement and washed twice⁵. Cells ($2.2-3.0 \times 10^7$), viable by trypan blue exclusion, were injected into recipient mice. In the first experiment the mice were then sensitized with 7% PCI in alcohol and contact sensitivity was assessed 6 d later by challenging the ears with 1% PCI in olive oil. The increase of ear thickness was then measured at 24 and 48 h³.

Table 1 The Inhibition of Contact Sensitivity by Lymph Node Cells from Unresponsive Mice: the Effect of Anti- θ "Serum" on the Suppressor Cells

Experiment	Lymph node cells transferred from mice injected with PSA (suppressor cells)	Treatment of suppressor cells	Increment of ear thickness ($\pm$ s.d.) at	
			24 h	48 h
1	None (positive control)		7.3 $\pm$ 0.69	6.8 $\pm$ 0.72
	None, unsensitized (negative control)		1.0 $\pm$ 0.57 *	1.1 $\pm$ 0.47 *
	2.2×10^7	None	2.2 $\pm$ 0.28 *	2.1 $\pm$ 0.32 *
	2.2×10^7	θ serum absorbed with AKR thymus	6.2 $\pm$ 1.18	6.1 $\pm$ 1.22
	2.2×10^7	θ serum absorbed with CBA thymus	3.4 $\pm$ 0.44 *	3.3 $\pm$ 0.33 *
2	None (positive control)		7.6 $\pm$ 1.49	
	None, unsensitized (negative control)		0.5 $\pm$ 0.20 *	
	3×10^7	None	2.6 $\pm$ 0.83 *	
	3×10^7	Unabsorbed θ serum	6.9 $\pm$ 0.33	
	3×10^7	θ serum absorbed with AKR thymus	6.2 $\pm$ 0.69	
	3×10^7	θ serum absorbed with CBA thymus	2.4 $\pm$ 1.06 *	

Donor mice received one (experiment 1) or five (experiment 2) injections of 5 mg picryl sulphonic acid. Suppressor lymph node cells either untreated, or treated *in vitro* with anti- θ "serum" followed by complement, were injected into recipients. The recipients were then sensitized with 7% picryl chloride and challenged with 1% picryl chloride 6 d later.

* Significantly different from the positive control by Student's *t* test at both 24 and 48 h ($P < 0.001$). None of the other figures were significantly different from the positive control ($P > 0.1$).

Table 2 The Inhibition of Passive Transfer of Contact Sensitivity by Lymph Node Cells from Unresponsive Mice: the Effect of Anti- θ "Serum" on the Suppressor Cells

Sensitized to picryl chloride (passive transfer cells)	Lymph node cells transferred from mice injected with PSA (suppressor cells)	Treatment of suppressor cells	Increment of ear thickness ($\pm$ s.d.) at	
			24 h	48 h
3.5×10^7	None (+ve control)		6.0 $\pm$ 0.57	5.1 $\pm$ 0.54
None	None (-ve control)		0.9 $\pm$ 0.36 *	0.9 $\pm$ 0.70 *
3.5×10^7	1.2×10^7	None	2.6 $\pm$ 0.63 *	2.4 $\pm$ 0.42 *
3.5×10^7	1.2×10^7	θ serum absorbed with AKR brain	5.8 $\pm$ 1.14	4.9 $\pm$ 1.18
3.5×10^7	1.2×10^7	θ serum absorbed with CBA brain	2.7 $\pm$ 0.59 *	2.5 $\pm$ 0.57 *

Passive transfer cells were obtained 4 days after painting with picryl chloride. Suppressor cells were obtained 5 days after a single injection of PSA. The suppressor cells were treated with anti- θ "serum" and C or left untreated, mixed with passive transfer cells and injected into normal recipients. The recipients were challenged immediately with picryl chloride.

* Significantly different from +ve control.

Table 3 Suppression of Contact Sensitivity by Suppressor Cells: Experiments in which both Donor and Recipient Mice were Irradiated and Restored with Thymus Cells ("T" Mice)

Suppressor cells transferred	Increment of ear thickness ($\pm$ s.d.) at	
	24 h	48 h
None (+ve control)	5.0 ± 0.2	5.6 ± 0.72
None, unsensitized (-ve control)	$0.3 \pm 0.13^*$	$0.5 \pm 0.17^*$
Lymph node (2×10^7)	$0.9 \pm 0.22^*$	$0.9 \pm 0.28^*$
Spleen (4×10^7)	$1.3 \pm 0.64^*$	$1.6 \pm 0.23^*$

Donor mice were irradiated at 900 R from a cobalt source. They were restored the same day with 7.5×10^7 normal thymus cells and injected the next day with 5 mg PSA. On day 5 their cells (suppressor cells) were transferred to recipients. The recipients were prepared by irradiation on day 4 and on day 5 received a mixture of suppressor cells and 6.0×10^7 normal thymus cells. The recipients were sensitized with picryl chloride on day 5 and challenged on day 11.

* Significantly different from positive control.

depress the passive transfer of contact sensitivity to irradiated "neutral" mice (that is, mice not restored with thymus cells) was tested. Both suppressor cells and the passive transfer cells were produced in "T" mice. Table 4 shows that suppressor cells depressed the passive transfer of contact sensitivity in "neutral" mice.

These results show that suppressor cells and passive transfer cells and their interaction occur in mice severely depleted of non-thymus derived lymphocytes⁷ and suggest that suppression of contact sensitivity can occur in the absence of B cells.

Suppressor T cells have been described in antibody systems including high dose unresponsiveness to haemocyanin (M. C. Cooper and G. L. Ada, unpublished) and allotypic suppression². The present results show that suppressor T cells also influence cell mediated immunity and that in this situation T cells act on T cells. Unpublished evidence shows that in

Table 4 Suppression of the Passive Transfer of Contact Sensitivity by Suppressor Cells: Experiments Using Irradiated and "T" Mice

Cells transferred from irradiated, thymus restored mice ("T" mice)		Increment of ear thickness in irradiated recipients ($\pm$ s.d.) at	
Sensitized to picryl chloride (passive transfer cells)	Injected with PSA (suppressor cells)	24 h	48 h
Lymph node (1.4×10^7)	— (+ve control)	4.7 ± 1.30	4.2 ± 0.99
—	— (-ve control)	$0.5 \pm 0.30^*$	$0.4 \pm 0.20^*$
Lymph node (1.4×10^7)	Lymph node (4×10^6)	$1.5 \pm 0.30^*$	$0.8 \pm 0.40^*$
Spleen (1.7×10^7)	— (+ve control)	3.4 ± 0.22	3.5 ± 0.60
Spleen (1.7×10^7)	Lymph node (5×10^6)	$0.8 \pm 0.47^*$	$0.4 \pm 0.28^*$
None	Lymph node (5×10^6)	$0.5 \pm 0.10^*$	$0.3 \pm 0.15^*$

* Significantly different from positive control.

Table 1 (experiment 1) shows that lymph node cells from donors given one injection of PSA depressed the ability of normal mice to develop contact sensitivity to PCI. Treatment of the suppressor cells *in vitro* with anti- θ "serum" and C virtually abolished this inhibition. Absorption of the anti- θ "serum" with CBA but not with AKR thymus made the serum inactive. Similar results were obtained when suppressor cells from mice given five injections of PSA were used (experiment 2).

Contact sensitivity to "oxazolone" can be passively transferred by lymph node cells taken 4 d after immunization and this transfer is a T cell phenomenon (G. L. A. and M. Z., unpublished). The injection of mixtures of suppressor and "passive transfer" cells into normal recipients provides a means of investigating whether suppressor cells block the effector stages of contact sensitivity. Table 2 shows that suppressor cells block the passive transfer of contact sensitivity to PCI. It also shows that the suppressor cells were ineffective after treatment with anti- θ "serum". Absorption of the "serum" with AKR and CBA brain confirmed its specificity for T cells.

The results obtained with anti- θ "serum" show that the depression of contact sensitivity by suppressor cells is due to T cells acting on a T dependent phenomenon; but these experiments do not exclude a subsidiary role of non-T lymphocytes. To investigate this question experiments were undertaken in irradiated mice restored with thymus cells ("T" mice).

Mice were irradiated at 900 R on day 0 and restored with normal thymus cells on the same day. Suppressor cells were produced by the injection of PSA on day 1 and the lymph node and spleen (suppressor) cells harvested on day 5.

The ability of these cells to depress contact sensitivity in other "T" mice was tested by injecting these suppressor cells into irradiated recipients restored with normal thymus cells. The recipients were then painted on the same day with PCI and challenged 6 d later. Table 3 shows that the suppressor cells depressed contact sensitivity under these circumstances. In a second experiment the ability of suppressor cells to

our system suppressor T cells do not interfere with the DNA synthesis which follows skin painting with picryl chloride (unpublished observation) but act instead at the effector stages of contact sensitivity.

We thank Mr Raymonde Robinson and Mrs Barbara Mayhew for assistance and Professor Gordon Ada for discussion.

M. ZEMBALA

Department of Experimental and Clinical Immunology,
Institute of Microbiology,
Medical School, Cracow

G. L. ASHERSON

Division of Immunology, Clinical Research Centre,
Watford Road, Harrow HA1 3UI, Middlesex

Received April 11, 1973.

- 1 Gershon, R. K., and Kondo, K., *Immunology*, **21**, 903 (1971).
- 2 Jacobson, E. N., Herzenberg, L., Riblet, R., and Herzenberg, L. A., *J. exp. Med.*, **135**, 1163 (1972).
- 3 Asherson, G. L., Zembala, M., and Barnes, R. M. R., *Clin. exp. Immunol.*, **9**, 111 (1971).
- 4 Reif, A. E., and Allen, M., *J. exp. Med.*, **120**, 413 (1964).
- 5 Asherson, G. L., and Ferluga, J., *Immunology* (in the press).
- 6 Greaves, M., and Raff, M. C., *Nature new Biol.*, **233**, 239 (1971).
- 7 Jacobson, H., and Blomgren, H., *Clin. exp. Immunol.*, **13**, 439 (1973).

Precision of Development in Chick Limb Morphogenesis

It is important for an understanding of pattern formation in development to know how precisely the patterns are specified. One type of mechanism may involve the specification of positional information in the form of some graded cellular parameter. This is interpreted by cells in terms of molecular differentiation according to their genome and past history. How precisely must positional information be specified or interpreted? To make the situation

more concrete, one can consider a line of 50 cells, the size of a typical positional field, and ask how accurately the position of any one could or need be specified. If one half were to be different from the other how accurately would the boundary be located? It is very difficult to obtain such data on the precision of development at the cellular level. One of the few situations where this type of information is available is in sea urchin development in which about 50 of the 1,000 cells of the blastula become primary mesenchyme. This process is rather imprecise with a coefficient of variation of about 10%² and corresponds with an overall impression of the lack of precision in sea urchin development^{1,3}. Maynard-Smith⁴ has provided an original discussion of continuous and quantized variation in adult structures but does not directly relate them to developmental processes. While geneticists have been conscious of what has been termed developmental noise⁵ or special environmental variance⁶, their studies, too, are not easily relatable to developmental processes at the cellular level. One approach is of particular interest, however, and that is the comparison of bilaterally symmetrical structures⁷, for differences between these could provide an estimate of the precision of development. Most studies of this type have been carried out on *Drosophila* and made use of numbers of bilateral structures such as bristles and ovarioles, though it has been briefly reported⁸ that the coefficient of variation of the ratio of wing lengths is 0.40%.

The embryonic chick limb can also be used to obtain such data on precision and these can be related to developmental processes at the cellular level⁹. We have compared the lengths of skeletal elements in left and right wings in embryos between the ninth and eleventh day of incubation. The data have been used both directly in the estimate of the precision of development and indirectly as an assay to judge the effect of various perturbations on the developing limb.

Forty embryos were killed between the ninth and eleventh days of incubation and fixed in 5% TCA. They were stained in 0.1% alcian green at a pH of 1.0, dehydrated and cleared in methyl salicylate. Cleared limbs were placed in pairs in a dish of methyl salicylate, then gently squashed flat under a piece of microscope slide. The limbs were examined and photographed under a Zeiss Stereo IV dissection microscope with transilluminator using a medical microscope side arm and 35 mm camera. Using a $\times 10$ micrometer eyepiece (interval unit = 50 μm with a "zoom" setting of 2.0) the lengths of some of the skeletal elements were measured to the nearest unit as shown (Fig. 1). It proved impossible to estimate the width of elements with similar precision. Two pairs of limbs were then selected and remeasured on successive days so as to obtain two estimates of the observer error. The length of the left element was subtracted from the right, and the number of times that a given difference was obtained (in steps of 1 unit = 50 μm) was recorded. Table 1 shows the mean

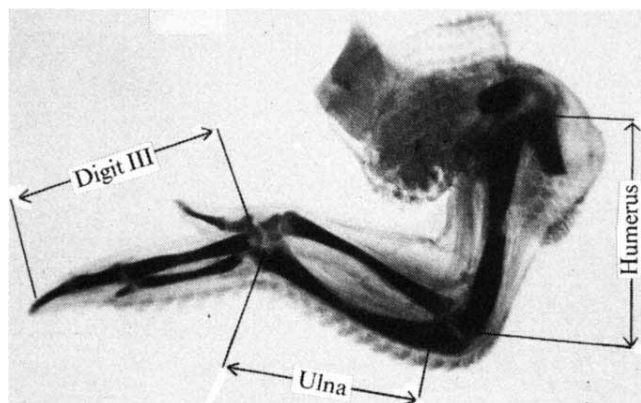


Fig. 1 Elements of chick limbs measured.

Table 1 Lengths of Skeletal Elements of Chick Embryo Limbs

Sample	Humerus	Ulna	Digit III
Mean difference	+0.04	-0.04	-0.40
Variance of difference	1.20	0.91	2.70
Mean length	83	70	80
Repeat measurements			
Mean difference	-0.5	0.0	-0.75
Variance of difference	0.86	0.86	1.07
Mean length	87	70	83
(One unit is equal to 50 μm)			

and variance of the difference between left and right elements, and the mean element length; it also shows the same values for repeat measurements of a single pair of limbs.

It is clear that there is little difference between the lengths of left and right skeletal elements in the wings of the same embryo. The random observer error as estimated by repeat measurements of the difference between wings in the same embryo is of the same order of magnitude, and so the real intrinsic difference between wings will be even less than the observed difference. From the means and standard deviations of our normal population sample in Table 1 we can give upper bounds to the intrinsic variability. We would expect that in at least 66% of a sample of normal embryos the difference in the length of left and right skeletal elements would be less than 50 μm , and in at least 99% of embryos less than 200 μm , for a total length of up to 5,500 μm . It is thus clear that the development of skeletal elements is remarkably precise.

We can attempt to relate that to the development at the cellular level. The ulna at 8-10 d is made up primarily of cartilage cells surrounded by matrix. We can estimate the number of cells along the proximo-distal axis by counting the number which touch an axial line transect; this comes to about 200. We can then compare this with a similar line transect through the presumptive ulnar region of a 4-d-old embryo⁹, along which we count about twenty cells. As the cross sectional area of the ulna does not appreciably increase between these two stages, it seems that the increase in length is due to each cell dividing on average about three times together with secretion of matrix. While errors and imprecision can occur at any stage of development, such as matrix secretion and cell division, errors in the specification of the initial primordium will be amplified more than later ones. If we ignore the possibility of there being length regulatory mechanisms at intermediate stages we are led to the conclusion that the error in initial specification of element length cannot be greater than 5%, that is, it is accurate to within one cell in twenty.

Apart from the intrinsic interest of any estimate of the precision of specification of form these results are also useful in assaying the effect of numerous perturbations of the chick limb. Studies or regulation of limb development can now be made quantitative instead of relying on morphological criteria such as the presence of normal or abnormal skeletal elements. If we choose the 99% confidence limit then the maximum permitted variation becomes: humerus $\pm 4\%$; ulna $\pm 4\%$; digit III $\pm 5\%$. Any difference between operated elements and contralateral control elements greater than these limits indicates a failure of the limb to regulate the length of the operated element. Such operations will be reported in detail elsewhere.

We thank Dr J. Lewis for his advice. This work is supported by the Science Research Council.

Note added in proof. We have also obtained evidence for the absence of a regulatory mechanism controlling overall length.

D. SUMMERBELL
L. WOLPERT

Department of Biology as Applied to Medicine,
The Middlesex Hospital Medical School, London W1

Received April 17, 1973.

- ¹ Wolpert, L., *Current Topics in Develop. Biol.*, **6**, 183 (1971).
- ² Hörstadius, S., *Wilhelm Roux. Arch. Entwickl. Org.*, **135**, 40 (1936).
- ³ Gustafson, T., and Wolpert, L., *Intern. Rev. Cytol.*, **15**, 139 (1963).
- ⁴ Maynard-Smith, J., *Proc. Roy. Soc., B*, **152**, 397 (1960).
- ⁵ Waddington, C. H., *Strategy of the Genes* (Allen and Unwin, London, 1957).
- ⁶ Falconer, D. S., *Introduction to Quantitative Genetics* (Oliver and Boyd, Edinburgh, 1960).
- ⁷ Mather, K., *Heredity*, **7**, 297 (1953).
- ⁸ Reeve, E. C. R., and Robertson, F. W., *J. Genet.*, **51**, 276 (1952).
- ⁹ Summerbell, D., Lewis, J., and Wolpert, L., *Nature New Biology* (in the press).

Growth Hormone Regulation by Melatonin and Serotonin

THERE is evidence to suggest the existence of a pineal gland substance which moderates growth both in man and the rat. In man, non-parenchymal tumours, such as gliomas or teratomas which result in destructive lesions of the pineal gland, are associated with precocious puberty^{1,2} and it has been hypothesized that such lesions prevent the pineal from secreting gonadal² and growth^{3,4} inhibitory factors. It was recently reported⁵ that the ability of the pineal gland to influence both growth and gonadal development may be due to two distinct physiological mechanisms. Rats which are blinded or kept in constant darkness show reduced body weight^{4,5}, reduced tibial length⁴, and reduced accessory organ weights⁴ as well as retarded puberty^{2,5,6}. Blinded rats were also found⁴ to have significantly reduced pituitary gland stores of growth hormone. The effects of blinding or constant darkness on growth and growth hormone stores were abolished by pinealectomy^{4,7}—a procedure also found⁸ to increase the gain of body weight in otherwise normal rats. Pinealectomy has also been reported to result in increased growth of experimental tumours in rats⁹. The observation¹⁰ of a diurnal fluctuation in the secretion of growth hormone in the rat further suggests that the lighting regime can modify this release. When lighting is reduced, concentrations of the pineal hormone melatonin (N-acetyl-*o*-methylserotonin) are increased because it is synthesized within the pineal gland from serotonin (5-hydroxytryptamine) by the action of the enzymes serotonin-N-acetyltransferase and hydroxyindole-*o*-methyltransferase, both of which exhibit their highest activities in the absence of light^{11,12}. The fact that the conditions favouring high melatonin production correlate with those which cause reduced growth (and the other way round) suggests that melatonin has an inhibitory role in growth hormone secretory mechanisms. Collu *et al.*¹³ demonstrated that intraventricular injections of serotonin stimulate secretion of growth hormone in the rat, and they proposed that in this animal secretion of growth hormone is controlled through serotonergic pathways. Serotonin has also been implicated as the stimulus for secretion of growth hormone after the onset of slow-wave (non-rapid eye movement, NREM) sleep in normal humans¹⁴. It thus seems that serotonin and its pineal derivative, melatonin, may have opposing effects on the secretion of growth hormone. We describe here experiments which support this contention.

Groups of non-gentled male and female Wistar rats, approximately 80 d old, were injected intraperitoneally with the following drugs or drug combinations dissolved in 0.5 ml physiological saline containing 20% (v/v) ethanol: 5-hydroxy-L-tryptophan (5-HTP, 10 mg kg⁻¹); 5-HTP (10 mg kg⁻¹) plus melatonin (80 mg kg⁻¹); 5-HTP (10 mg kg⁻¹) plus cyproheptadine (10 mg kg⁻¹). Thirty minutes later the rats were decapitated and their blood was collected. The serum level of radioimmunoassayable (RIA) growth hormone of each rat was determined using materials supplied by the National Institutes of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program, Bethesda. The results are shown in Table 1.

The doses of 5-HTP and melatonin were chosen on the basis of possible competition for hypothalamic receptor sites mediating in the release of growth hormone releasing hormone. The doses of the compounds required to elevate brain concentrations to a suitable level were assessed from the results of Okada *et al.*¹⁵ for 5-HTP, and those of Anton-Tay and Wurtman¹⁶ for melatonin. The dose of melatonin thus chosen would be expected to increase the hypothalamic melatonin concentration by about 1 µg g⁻¹, that is, sufficient to compete with the increased serotonin concentration due to 5-HTP. The serotonin antagonist cyproheptadine¹⁷ was tested at the same dose level as that used for 5-HTP.

The results show 5-HTP to be a potent stimulus for secretion of growth hormone in the rat. The effect is presumably due to the increased levels of brain serotonin^{15,18} following decarboxylation of 5-HTP. This finding is in accord with the results of Collu *et al.*¹³ for intraventricular serotonin. That secretion of growth hormone in the rat is mediated through (presumably hypothalamic) serotonin receptors is supported by the result for the cyproheptadine treatment which inhibited the growth hormone response to 5-HTP. The results also show that melatonin can block the growth hormone response to 5-HTP. This, we think, is a significant finding and could account, in part at least, for the growth attenuating abilities of the pineal system mentioned above. Laborit¹⁴ has proposed that serotonin release is responsible for the secretion of human growth hormone which occurs with slow-wave (NREM) sleep¹⁹. Significantly, it has been observed²⁰ that blind human subjects show no slow-wave sleep-induced secretion of growth hormone. Increased hours of daylight and light intensity resulting in decreased melatonin production could explain the observation that children grow more rapidly in summertime and in dry seasons^{21,22}. Our findings could also explain the results of Starr²³ who reported decreased serum growth hormone levels in cancer patients after they had been treated with melatonin. The precise mechanism by which melatonin blocks serotonin-induced growth hormone release remains to be elucidated. The ability of the pituitary gland of the cat to concentrate peripherally administered melatonin²⁴ suggests the possibility of a direct action of melatonin on the pituitary to block release of growth hormone. However, by analogy with the catecholamines where *o*-methylation of dopamine, for example, results in effective hypothalamic dopamine receptor-site blockers which competitively block dopamine inhibited prolactin release²⁵ it seems likely that melatonin, being an *o*-methylated derivative of serotonin, could act as a serotonin receptor-site antagonist at the level of the hypothalamus. Anton-Tay *et al.*²⁶ have shown that intraperitoneal administration of melatonin can lead to increased levels of serotonin in the midbrain and hypothalamus of the rat. As possible reasons for this finding, they suggested²⁶ that melatonin can inhibit the release and/or metabolism of

Table 1 Stimulation and Blockade of Rat Growth Hormone Secretion

Group	I	II	III	IV
Treatment	Controls (solvent only)	5-HTP	5-HTP + melatonin	5-HTP + cyproheptadine
Number of animals	10	14	13	6
Serum GH level, ng ml ⁻¹ (mean ± s.e.m.)	7.4 ± 0.68	101.1 ± 14.5	5.9 ± 2.12	4.2 ± 1.83
Significance compared with group I	—	P < 0.0005	NSD	P < 0.05
Significance compared with group II	P < 0.0005	—	P < 0.0005	P < 0.0005

GH, growth hormone; NSD, no significant difference.

serotonin. This, together with our data, supports the concept that melatonin can act as a general blocker, or competitive inhibitor, at serotonin receptor sites. We propose that such an action is responsible for the opposing effects of melatonin and serotonin on growth hormone secretion and indicates one mechanism by which the pineal gland may regulate growth.

This work was supported in part by a grant from the National Health and Medical Research Council of Australia.

G. A. SMYTHE
L. LAZARUS

Garvan Institute of Medical Research,
St Vincent's Hospital,
Sydney 2010

Received February 26; revised April 13, 1973.

- ¹ Kitay, J. I., and Altschule, M. D., in *The Pineal Gland* (Harvard University Press, Cambridge, 1954).
- ² Cohen, R. A., Wurtman, R. J., Axelrod, S., and Snyder, S., *Ann. Intern. Med.*, **61**, 1144 (1964).
- ³ Wiener, H., *New York State J. Med.*, **68**, 1019 (1968).
- ⁴ Sorrentino, S., Schalch, D. S., and Reiter, R. J., in *Growth and Growth Hormone* (edit. by Pecile, A., and Muller, E. E.) (Excerpta Medica, Amsterdam, 1972).
- ⁵ Osman, P., Welschen, R. W., and Moll, J., *Neuroendocrinology*, **10**, 121 (1972).
- ⁶ Wurtman, R. J., Axelrod, J., and Kelly, D. E., in *The Pineal* (Academic Press, New York, 1968).
- ⁷ Eayrs, S. T., and Ireland, K. F., *J. Endocrinol.*, **25**, 1470 (1949).
- ⁸ Malm, O. J., Skaug, O. E., and Lingjoerde, P., *Acta Endocrinol.*, **30**, 22 (1959).
- ⁹ Rodin, A. E., *Cancer Res.*, **23**, 1545 (1963).
- ¹⁰ Muller, E. E., Giustina, G., Miedico, D., Pecile, A., Cocchi, D., and King, F. W., *Proc. Soc. Exp. Biol. Med.*, **135**, 934 (1970).
- ¹¹ Klein, D. C., and Weller, J. L., *Science*, **196**, 1093 (1970); **177**, 532 (1972).
- ¹² Wurtman, R. J., Axelrod, J., and Phillips, L. S., *Science*, **142**, 1071 (1963).
- ¹³ Collu, R., Fraschini, F., Visconti, P., and Martini, L., *Endocrinology*, **90**, 1231 (1972).
- ¹⁴ Laborit, H., *Res. Commun. Chem. Pathol. Pharmacol.*, **3**, 51 (1972).
- ¹⁵ Okada, F., Saito, Y., Fujieda, T., and Yamashita, I., *Nature*, **238**, 355 (1972).
- ¹⁶ Anton-Tay, F., and Wurtman, R. J., *Nature*, **221**, 474 (1969).
- ¹⁷ Stone, C. A., Wenger, H. C., Ludden, C. T., Starvoski, J. M., and Ross, C. A., *J. Pharmacol. Exp. Ther.*, **131**, 73 (1961).
- ¹⁸ Davidson, J., Sjoerdsma, A., Loomis, L. N., and Udenfriend, S., *J. Clin. Invest.*, **36**, 1594 (1957).
- ¹⁹ Sassin, J. F., Parker, D. C., Mace, J. W., Gotlin, R. W., Johnson, L. C., and Rossmann, L. G., *Science*, **106**, 513 (1969).
- ²⁰ Krieger, D. T., and Glick, S., *J. Clin. Endocrinol. Metab.*, **33**, 847 (1971).
- ²¹ Thompson, D. A., in *Growth and Form* (Cambridge University Press, 1942).
- ²² Tanner, J. M., in *Growth at Adolescence*, second ed. (Blackwell, Oxford, 1962).
- ²³ Starr, K. W., in *Progress in Clinical Cancer* (edit. by Ariel, I. M.), IV (Grune and Stratton, New York, London, 1970).
- ²⁴ Wurtman, R. J., Axelrod, J., and Potter, L. T., *J. Pharmacol. Exp. Ther.*, **143**, 314 (1964).
- ²⁵ Smythe, G. A., and Lazarus, L., *Endocrinology* (in the press).
- ²⁶ Anton-Tay, F., Chou, C., Anton, S., and Wurtman, R. J., *Science*, **162**, 277 (1968).

Evidence for Pheromone-influenced Homing by Migrating Atlantic Salmon, *Salmo salar* (L.)

THERE have been many observations on the homing of adult salmonids to the river, and even part of river, of their birth and numerous attempts to elucidate the navigational cues used to locate and identify the freshwater home. In 1880, Buckland¹ suggested that each river had a characteristic smell, and that olfaction played an important part in home-stream recognition. Homing by olfaction has since been demonstrated conclusively in Pacific salmon of the genus *Oncorhynchus*²⁻⁶, and neurophysiological evidence for the role of olfaction presented by Hara *et al.*⁷. Evidence is presented here which indicates that the homing of adult Atlantic salmon may be

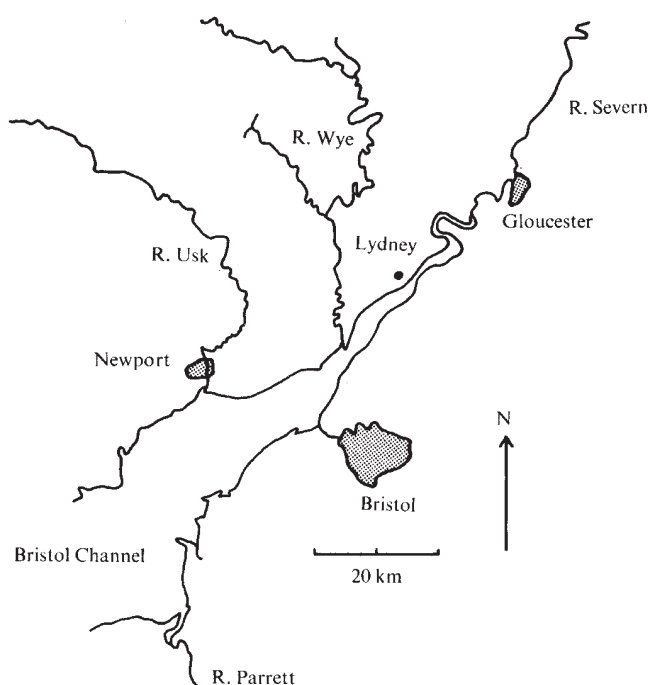


Fig. 1 The upper Bristol Channel.

largely dependent on the presence of other individuals in the river.

The suggestion that the homing of adult salmonids may be influenced by pheromones or the metabolic products of a specific population is not new; Chidester⁸ mentioned the possibility, but concluded that it was rather unlikely. White⁹ described experiments in which Atlantic salmon fry were planted in a stream previously containing no salmon; later that year a run of adult salmon occurred in that stream. Miles¹⁰ found that elvers were attracted to streams containing adult eels, and that the presence of other elvers reduced the attractiveness. He also found that other odours played an important role. Nordeng¹¹ put forward evidence to indicate that the homing of a migratory population of the char, *Salvelinus alpinus*, in Norway was influenced by pheromones and suggested that the attractant might be secreted in the mucus. In a study of EEG activity in migrating chinook salmon (*O. tshawytscha*), Oshima *et al.*¹² observed that a non home-stream water gave an increased olfactory response after two adults had been kept overnight in the water to be tested. Adults of another species of the same genus did not produce this effect.

Between 1958 and 1964 more than 50,000 smolts were tagged in the Rivers Usk, Wye and Severn (the three main rivers draining into the upper Bristol Channel) (Fig. 1) by the Ministry of Agriculture, Fisheries and Food (MAFF) (Swain, personal communication) and the River Boards¹³⁻¹⁵. Of 104 marked adults caught in subsequent years in these rivers above the tidal limits, ninety-six were taken in the river in which they were marked, representing a 92% homing accuracy between the three rivers. The pattern of recaptures in the tidal reaches is of particular interest. In the Severn estuary between Lydney (16 and 40 km above the mouths of the Wye and the Usk) and the tidal limit at Gloucester the recapture of marked adults was as follows—fifteen Severn fish, nine Wye fish and five Usk fish. Considering the number of smolts marked in each river, this represents an unbiased sample of fish from the three rivers.

This pattern suggests a searching and overshooting behaviour and could be interpreted as indicating that salmon are attracted in the first instance to freshwater in general, being able to discriminate between streams only when very close to, or even in, the estuary. If this were so, however, one would expect a greater wandering to occur between estuaries in other

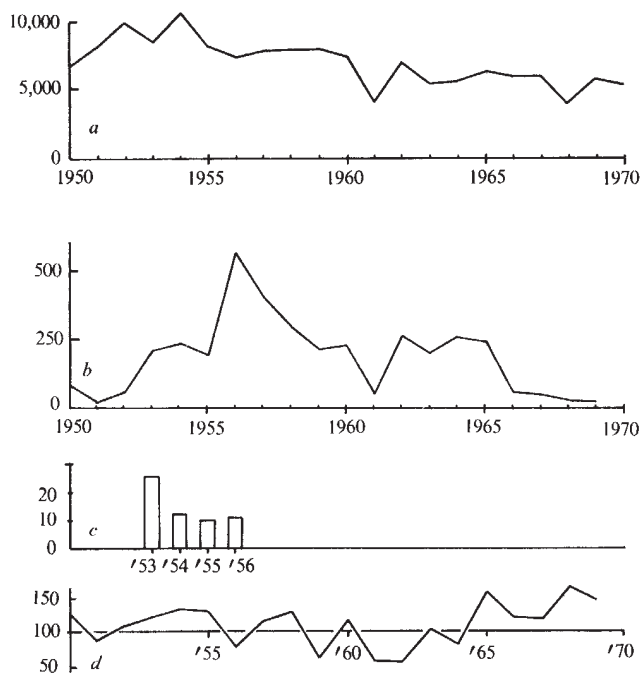


Fig. 2 a, Annual commercial catch of salmon for the Usk-Wye-Severn tidal fishery; b, annual commercial catch for the Parrett estuary fishery; c, numbers of salmon ova planted (thousands) in the River Tone, a tributary of the Parrett; d, Rainfall in May, June and July in the Somerset River Board area, expressed as a percentage of the normal.

areas than is observed; in general few salmon are reported in the estuaries of non-salmon rivers. Of 23,000 smolts tagged by MAFF on the River Coquet in Northumberland in 1952-57, only six were caught as adults in the non-tidal reaches of other rivers, all salmon streams. These included three in the Tweed and one each in the Tay, Aln and Yorkshire Esk¹⁶. Many were caught by netsmen off other neighbouring salmon rivers. Most wanderers from the River Axe—where MAFF attempts to mark all descending smolts—are recorded in other salmon rivers (Swain and Champion, manuscript in preparation). The straying of adult salmon into a non-salmon river is usually apparent from the presence of dead specimens or by accidental capture.

I suggest that the presence of salmon (parr?) in a river renders its estuary attractive to migrating adults in the first instance, and that a metabolic product of a discrete population could be the odour to which adults home. Some evidence for this comes from the River Parrett in Somerset, whose estuary enters the Severn estuary on the opposite side to those of the Usk and Wye (Fig. 1). Even though no salmon ascended the river to spawn, there has been a small commercial salmon fishery on the estuary for many years. It seems that adults returning to the Usk, Wye and Severn stray into the estuary. The annual catches for the fishery, representing a fairly constant fishing effort, are shown in Fig. 2^{17,18}. In 1953 about 25,000 salmon ova were planted by the Somerset River Board in the River Tone, a tributary of the Parrett, and about 10,000-12,000 in each year from 1954 to 1956¹⁷. These ova were obtained from a variety of sources in Iceland, North Wales and Scotland. During the summer of 1953 (a few months after the first egg planting) the estuary catch was the highest for several years. It remained high for several years, and in 1956 was considered to be the best ever.

Figure 2 also shows the total commercial catches of the Usk-Wye-Severn fishery, indicating that the increased Parrett catch was not merely a reflexion of increased numbers of salmon in all areas, and an index of rainfall for the 3 months of main catches. There is no correlation between rainfall and catch. In 1954 and for some years following, a few adults ascended the River Tone, successfully spawning at least once¹⁷.

The last adult salmon were reported in the Tone in 1964, and in 1966 the estuary catch fell to a level below that typical of the period before the stocking programme. Most of the fish caught in the estuary during the high-catch years were probably not Tone fish: the first year was too soon after the stocking; the numbers of adults would have represented an extraordinarily high return for such small numbers of ova planted; and between 1962 and 1965 several fish bore tags of the Usk-Wye-Severn experiment discussed above. Of 943 adults caught in these 4 yr, eight had been tagged as smolts in the Severn, three in the Wye and four in the Usk. These proportions are not significantly different from the proportions of tagged fish in the Usk-Wye-Severn fishery.

Although this evidence is only circumstantial, it does seem that, during the years when a tributary contained a population of salmon parr from a stocking programme and later a small spawning run, increased numbers of salmon were attracted to the estuary of the River Parrett. It is probable that few of the fish in the estuary had originated in the River Tone, and that few actually ascended beyond the tidal limit.

Assuming the attractant has a molecular weight similar to that of an extract of mammalian skin repellent to salmon studied by Alderdice *et al.*², and using their threshold values, the total weight in the Parrett estuary need only be in the order of 1 g d⁻¹. If an olfactory acuity comparable to that of eels¹⁹ is involved, a considerably smaller quantity would suffice.

I thank Mr A. Swain of the MAFF Salmon and Freshwater Fisheries Laboratory for his help and allowing access to unpublished data.

D. J. SOLOMON

*Salmon and Freshwater Fisheries Laboratory,
Ministry of Agriculture, Fisheries and Food,
Whitehall Place, London SW1A 2HH*

Received February 15; revised March 14, 1973.

- Buckland, F., *Natural History of British Fishes* (Unwin, London, 1880).
- Alderdice, D. F., Brett, J. R., Idler, D. R., and Fagerlund, U., *Fish. Res. Bd Can., Prog. Rept. Pacific Coast Sta.*, **98**, 10 (1954).
- Wisby, W. J., and Hasler, A. D., *J. Fish. Res. Bd Can.*, **11**, 472 (1954).
- Idler, D. R., McBride, J. R., Jones, R. E. E., and Tomlinson, N., *Can. J. Biochem. Physiol.*, **39**, 1575 (1961).
- Fagerlund, U. H. M., McBride, J. R., Smith, M., and Tomlinson, N., *J. Fish. Res. Bd Can.*, **20**, 1457 (1963).
- Hasler, A. D., *Underwater Guideposts* (University of Wisconsin, Madison, 1966).
- Hara, I. J., Veda, K., and Gorbman, A., *Science, N.Y.*, **149**, 884 (1965).
- Chidester, F. E., *J. exp. Biol.*, **2**, 79 (1924).
- White, H. C., *Trans. Am. Fish. Soc.*, **64**, 360 (1934).
- Miles, S. G., *J. Fish. Res. Bd Can.*, **29**, 1591 (1968).
- Nordeng, H., *Nature*, **233**, 411 (1971).
- Oshima, K., Hahn, W. E., and Gorbman, A., *J. Fish. Res. Bd Can.*, **26**, 2111 (1969).
- Severn River Board, *Annual Reports* (1959-1965).
- Usk River Board, *Annual Reports* (1959-1965).
- Wye River Board, *Annual Reports* (1959-1965).
- Northumberland and Tyneside River Board, *Annual Reports* (1953-1960).
- Somerset River Board, *Annual Reports* (1951-1965).
- Somerset River Authority, *Annual Reports* (1966-1970).
- Teichmann, H., *Naturwissenschaften*, **44**, 242 (1957).

New Bioassay for Luteinizing Hormone

UNTIL now it has not been possible to measure, by biological assay, the basal circulating concentrations of luteinizing hormone (LH). As a result, all information on the physiological role of this critically important pituitary glycoprotein is based either on indirect evidence, or on the use of immunological assays which do not necessarily measure biologically active hormone. We now describe a specific biological assay

for LH which is more sensitive than radioimmunoassay, and several orders of magnitude more sensitive than other biological assays. The principle of the new method is based on a combination of the ovarian ascorbic acid depletion (OAAD) technique of Parlow¹, and the histochemical method of measuring ACTH described by Chayen *et al.*². Reducing activity in ovarian slices treated with LH is estimated by microdensitometry after staining with Prussian blue.

Female weanling Wistar rats (21–25 d old) received a subcutaneous injection of 50 IU of pregnant mares' serum gonadotrophin ('Gestyl', Organon) followed 50–60 h later by 25 IU of human chorionic gonadotrophin ('Pregnyl', Organon). Seven to 10 d after the first injection the rats were killed and the ovaries removed, stripped of surrounding fat and each cut into three equal pieces. Each portion was placed on defatted lens paper on a stainless steel mesh grid in a vitreosil dish containing Trowell's T-8 medium (pH 7.6) enriched with 10^{-3} M sodium ascorbate. The dishes were placed in an air-tight perspex culture chamber and incubated for 5 h at 37° C in 95% oxygen and 5% CO₂. The medium was then removed and replaced with T-8 medium containing human LH or dilutions of plasma samples. After exposure for 4 min to LH or plasma the tissue was removed and chilled rapidly by immersion in *n*-hexane maintained at –65° C by a mixture of solid carbon dioxide and industrial spirit. After freezing, the ovarian tissue was placed in a cold glass tube and stored at –70° C. Sections of tissue 12 μ m thick were prepared in a cryostat at –30° C. After drying, the sections were stained by immersion in a mixture of 2.4% ammonium ferric sulphate (3 vols) and 0.1% potassium ferricyanide (1 vol). Fresh stain was added three times at intervals of 7 min to give a total reaction time of 21 min. The intensity of colour produced by the formation of ferric ferricyanide (Prussian blue) in the theca lutein cells, which is a function of the redox potential of the tissue, was then measured by scanning and integrating microdensitometry using the 'Vickers' M85 microdensitometer. The integrated extinction of light in ten areas (approximately twenty cells per area) of luteinized tissue was measured at 640 nm and again at 460 nm to correct for non-specific absorption of light by the section; the difference between the two readings was calculated and the result multiplied by 100 for convenience.

A graph of the integrated extinction (times 100) against the concentration of LH (MRC 68/40) revealed an inverse linear correlation between the intensity of the stain in the theca lutein cells and the logarithm of the concentration of LH over the range 5–0.005 mU ml⁻¹ (Fig. 1). The mean index of precision calculated from sixteen consecutive assays performed by two assayists was 0.12. The mean slope of the regression line for sixteen assays was 1.96, that is, for each ten-fold change in LH concentration a change of 1.96 units of extinction occurred. The minimum detectable quantity of LH (MRC 68/40) was 0.005 mU ml⁻¹ of medium.

A variety of natural and synthetic protein and peptide hormones was studied to assess the specificity of the assay (Table 1). These were purified human FSH (CPDS/3), ovine prolactin (NIH-P-S8), purified porcine ACTH (third IWS), synthetic α (1–24) ACTH ('Synacthen', Ciba), synthetic AVP (Ferring AB) and purified human TSH (MRC DE 323). All

Table 1 Cross-reactivity of Various Peptide Hormones in the LH Redox Bioassay

Peptide	Percentage cross-reactivity
FSH (CPDS/3)	1.0
Ovine prolactin (NIH-P-S8)	1–2
ACTH (third IWS)	0.3
ACTH ('Synacthen')	<0.01
AVP (synthetic)	<0.01
TSH (DE 323)	1.0

All hormones were assayed at two concentrations, 100 and 10 ng ml⁻¹, against LH IRC 2/64.

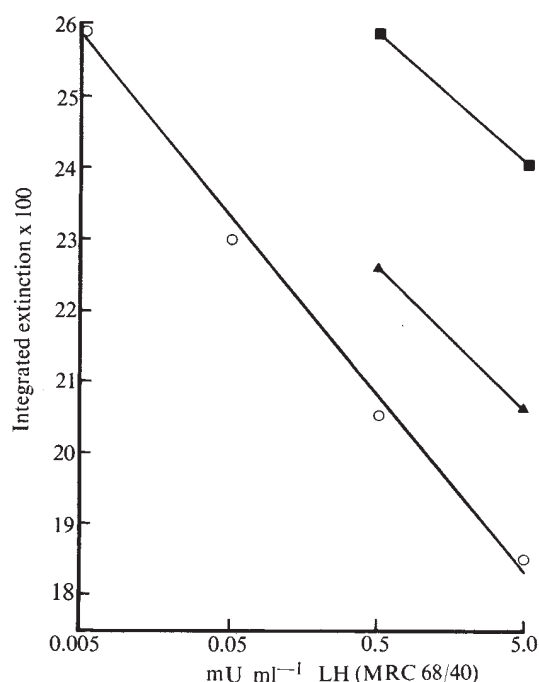


Fig. 1 LH redox standard curve: ○, standard LH; ▲, normal male; plasma diluted 1:10 and 1:100; ■, isolated gonadotrophin deficiency; plasma diluted 1:2.3 and 1:23.

were tested at two concentrations of 100 and 10 ng ml⁻¹ and compared with human LH (IRC 2/64) on a weight basis. This LH preparation was chosen for these comparisons because of its high degree of purity and low content of FSH and TSH as determined by radioimmunoassay. The FSH and ovine prolactin preparations cross-reacted to the extent of 1–2%, which could be caused by contamination with LH as a similar cross-reaction was observed in a specific radioimmunoassay. Similarly, LH contamination may account for the small degree of cross-reactivity (0.3%) of purified porcine ACTH (third IWS), as biologically active synthetic α (1–24) ACTH gave no cross-reaction (<0.01%).

A comparison between two different preparations of LH showed that MRC LH 69/104 was approximately twice as potent as MRC LH 68/40, assuming a content of 25 IU of 69/104 and 40 IU 68/40 per ampoule. This is within the limits of reported potency estimates for these materials³. The preparation LH IRC2/64, used in the specificity studies, gave a potency estimate of 4,000 IU mg⁻¹ when assayed against MRC 68/40.

Table 2 Comparison of Plasma LH Concentrations (mU ml⁻¹, MRC 68/40) Measured by the Redox Bioassay and Radioimmunoassay

Diagnosis	Redox bioassay	Radio-immunoassay
Normal male	5.1	2.9
Post-menopausal female	23	20.2
Isolated gonadotrophin deficiency	0.07	<0.4
Normal male after LHRH	40	44.1

Plasma LH concentrations in a normal male before and after administration of 100 μ g luteinizing hormone-releasing hormone (LHRH), a post-menopausal female, and a man with apparent isolated gonadotrophin deficiency were measured by both the redox bioassay and radioimmunoassay (Table 2). The radioimmunoassay could not detect LH in the plasma of the patient with presumed isolated gonadotrophin deficiency (<0.4 mU ml⁻¹), although a concentration of 0.07 mU ml⁻¹ was measured by the redox bioassay. The other concentrations were in good agreement.

The specificity of this assay is at least equivalent to that of any existing system for measurement of LH. The

sensitivity is better than that of a typical radioimmunoassay, and vastly superior to that of the best current bioassays. Concentrations of LH can be estimated which have been previously undetectable by other assay techniques, and it will be possible for the first time to determine biologically active hormone throughout the menstrual cycle. It thus appears that the type of histochemical assay, initially described by Chayen and his colleagues for ACTH, is applicable to other hormones and may represent a new dimension in endocrinological investigation.

The hormone preparations used in these studies were provided by the MRC Division of Biological Standards, Dr Anne Hartree and Dr W. Butt. These studies were supported by the Wellcome Trust, R. K. is supported by the MRC and I. M. H. by the MRC (New Zealand).

LESLEY H. REES
I. M. HOLDAWAY
ROSANNE KRAMER
A. S. MCNEILLY
T. CHARD

Departments of Chemical Pathology and
Obstetrics and Gynaecology,
St Bartholomew's Hospital,
London EC1

Received March 20, 1973.

¹ Parlow, A. F., *Fedn Proc.*, **17**, 402 (1958).

² Chayen, J., Loveridge, N., and Daly, J. R., *Clin. Endocrinol.*, **1**, 219 (1972).

³ Bangham, D. R., and Borth, R., *Acta Endocrinol.*, **71**, 625 (1972).

Ethanol and Methanol Metabolites in Alcohol Withdrawal

THE twenty-one subjects participating in this study were serial admissions to the St Louis Detoxification Center during the period of December 2-21, 1971. Their ages ranged from 29 to 72 yr and they comprised twenty males and one female. A blood sample (10 ml) was obtained from the patient on admission, when a symptom intensity rating evaluation was carried out^{1,2}. Blood concentrations of ethanol, methanol, acetaldehyde and formaldehyde-formate were determined by gas-liquid chromatography^{3,4}. This procedure was repeated on four consecutive days (total of five samples), after which the patient was discharged from the Center.

All participating subjects received chlordiazepoxide, flurazepam and a high potency vitamin B complex and C in a fixed schedule^{5,6}.

About one-half of the subjects had initial blood levels of ethanol of less than 100 mg/100 ml, while those of the remainder were well above 100 mg/100 ml. We therefore arbitrarily selected a blood level of ethanol of 100 mg/100 ml as the criterion for categorizing the patients into two groups—a low-alcohol group and a high-alcohol group. Students *t*-test or the *t*-test for the difference of matched pairs was used for the statistical evaluation of the data⁷.

The mean admission blood alcohol level of the low-alcohol group was 14.4 mg/100 ml (s.e. ± 7.1) while that of the high-alcohol group was 309 mg/100 ml (± 27.2). The blood ethanol levels for both groups over the 5-d observation period (Fig. 1) rapidly returned towards a non-alcoholic level, although on days 1 and 2 they were still significantly higher in the high-alcohol group.

Blood acetaldehyde concentrations over the 5 withdrawal days for the two groups are shown in Fig. 1. The high-alcohol group acetaldehyde blood level was significantly higher on admission day (0.747 ± 0.162 mg/100 ml) than it was in the low-alcohol group (0.378 ± 0.080 mg/100 ml). This large and statistically significant difference between the two groups endured for the 5 d of hospital confinement. These blood

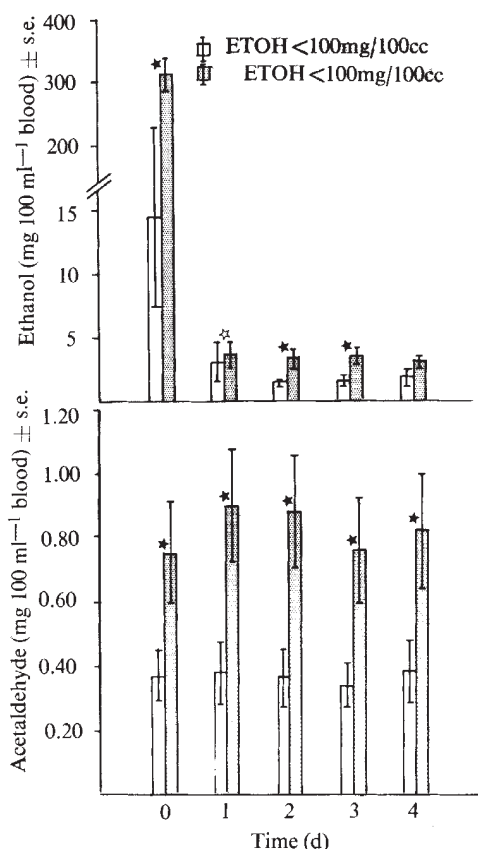


Fig. 1 Upper: Blood ethanol levels and standard errors in high-alcohol ($N=12$) and low-alcohol ($N=9$) separated groups during alcohol withdrawal. ☆: $P \leq 0.05$ between groups on common measurement day; * $P \leq 0.05$ between each group on different measurement days. Lower: Blood acetaldehyde levels and standard errors as above.

acetaldehyde concentrations, particularly for the high-alcohol group, are considerably higher than those reported in a laboratory controlled alcohol intake-withdrawal study⁸. But, as suggested from that study and supported by some of our laboratory observations, the 5-10-fold increased blood acetaldehyde concentrations in our patients could be a reflexion of the content of impurities contained in the alcoholic beverages consumed by our patients when "on the street".

The blood methanol concentration (Fig. 2) also exhibited marked differences between the two groups. There was a highly significant elevation of methanol in the admission sample for the high-alcohol group (1.54 ± 0.27 mg/100 ml) which was also apparent on day 1 (0.17 ± 0.08 mg/100 ml).

The formaldehyde-formic acid blood concentrations for the high-alcohol group were higher on admission and for each of the 4 observation days (Fig. 2). Peak level (5.58 ± 1.18 mg/100 ml) achieving significance was reached on the third observation day, and this significant elevation over the formaldehyde-formic acid blood concentration in the low-alcohol group (1.20 ± 0.17 mg/100 ml) was still observed on the fourth observation day. To our knowledge, this formaldehyde-formic acid response has not been reported before from this type of clinical population.

Both the high- and the low-alcohol groups exhibited high (abnormal) total scores in the intensity of withdrawal symptoms rating scores obtained on the admission day evaluation (Fig. 3). Subsequent daily behavioural evaluations showed that the rating scores for total withdrawal symptoms of the low-alcohol group rapidly returned towards normal, the total scores on all days being significantly lower than the admission score. In the high-alcohol group, the abnormal behaviour was greatest on this first observation day and then gradually returned towards normal levels. Examination of the peak scores for each of the ten factors revealed that only eating and sleeping disturbance

differed significantly between the high- and low-alcohol groups, the more deviant scores belonging to the high-alcohol group. In all cases, however, the high-alcohol group had statistically significant elevations in blood concentrations of ethanol, methanol and their metabolites and also showed total disrupted withdrawal signs and symptoms significantly greater than those of the low-alcohol groups.

Separating the twenty-one subjects participating in this study into high- and low-alcohol groups resulted in the emergence of a number of interesting differences between the two groups. The delayed and marked elevation in blood concentrations of the methanol metabolites, formaldehyde-formate, does support the hypothesis that the oxidative enzyme systems induced by chronic alcohol consumption will shift to metabolize methanol when ethanol is abruptly withdrawn. This would be expected and is consistent with the concept of competitive inhibition for the common enzyme systems for methanol and ethanol metabolism. Increased availability of the common enzyme systems shortly after abrupt ethanol withdrawal was thus associated with a marked decrease in methanol blood level and a delayed and marked elevation in the blood concentration of the methanol metabolites, formaldehyde-formate. We speculate that a critical accumulation of these methanol metabolites may be the primary factor which results in the expression of withdrawals.

These changes in formaldehyde-formate levels, together with the observed high and sustained acetaldehyde blood levels (high-alcohol group) implicated the impurities contained in the alcoholic beverages consumed by the "street alcoholics" before admission. The much higher blood acetaldehyde levels reported for "street alcoholics" used in this study compared with levels obtained from alcoholics in a controlled laboratory indicates that caution must be exercised in the interpretation, extra-

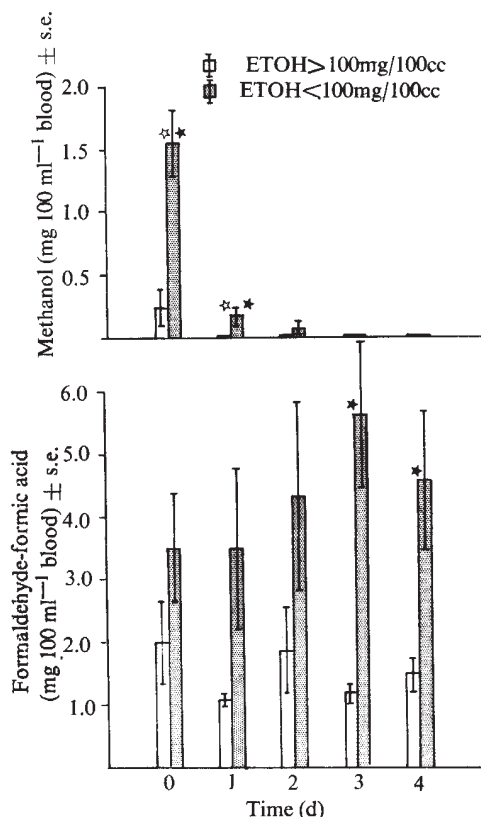


Fig. 2 Upper: Blood methanol levels and standard errors in high-alcohol ($N=12$) and low-alcohol ($N=9$) separated groups during alcohol withdrawal. ★: $P \leq 0.05$ between groups on common measurement day. ☆: $P \leq 0.05$ between each group on different measurement days. Lower: Blood formaldehyde-formic acid levels and standard errors as above.

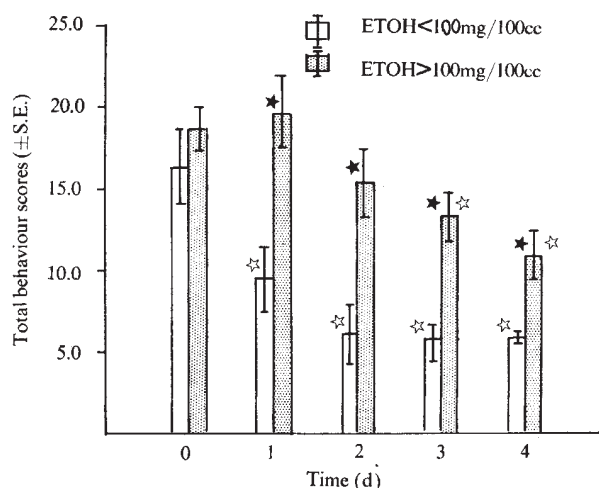


Fig. 3 Severity of behavioural disruption scores for high-alcohol ($N=12$) and low-alcohol ($N=9$) separated groups during alcohol withdrawal. ★: $P \leq 0.05$ between groups on common measurement days; ☆: $P \leq 0.05$ between each group on different measurement days.

polation or application of the results obtained in the artificial setting to what is happening in the "real world". This consideration as well as the many speculations and questions raised will be examined further in subsequent studies.

GASTON MAGRINAT*
JOHN P. DOLAN
RALPH L. BIDDY
LOWELL D. MILLER†
BERNARD KOROL

St Louis VA Hospital and Department of Psychiatry,
St Louis University School of Medicine,
St Louis, Missouri

Received March 8; revised April 30, 1973.

* Present address: VA Hospital, 1201 NW 16th Street, Miami, Florida 33125.

† Present address: Marion Laboratories, Kansas City, Missouri.

- ¹ Gross, M. M., Rosenblatt, S. M., Chartoff, S., Herman, A., Schacter, M., Sheinkin, D., and Broman, M., *Quart. J. Stud. Alcohol*, **32**, 611 (1971).
- ² Gross, M. M., Eastlyn, L., Meena, N., and Hastey, S. M., in *Biology of Alcoholism*, 3 (edit. by Kissin, B., and Begleiter, H.) (Plenum, New York, in the press).
- ³ Steinberg, M., *J. Foren. Sci.*, **10**, 201 (1965).
- ⁴ Goldbaum, L. R., *J. Foren. Sci.*, **9**, 63 (1964).
- ⁵ Kaim, S. C., Klett, C. S., and Rothfeld, B., *Amer. J. Psych.*, **125**, 12 (1969).
- ⁶ Klett, C. S., Hollister, L. E., Caffey, jun., E. M., and Kaim, S. C., *Arch. Gen. Psych.*, **24**, 174 (1971).
- ⁷ Snedecor, G. A., *Statistical Methods*, 106 (Iowa State College G.W. Press, Ames, Iowa, 1955).
- ⁸ Majchrowicz, E., and Mendelson, J. H., *J. Pharmacol. Exper. Therap.*, **179**, 298 (1971).

Isolation and Characterization of Agent causing Swim Bladder Inflammation in Carp

INFECTIOUS swim bladder inflammation (aerocystitis) is a contagious disease of carp (*Cyprinus carpio*) causing severe losses in carp culture. The aetiology of the disease, which is characterized by pathological changes mainly of the swim bladder, has been widely debated. Various bacteria, especially *Pseudomonas* sp., *Aeromonas* sp., and *Micrococcus* sp., have been regarded as causative agents¹⁻³ but all attempts to reproduce the disease with representative bacterial strains have failed.

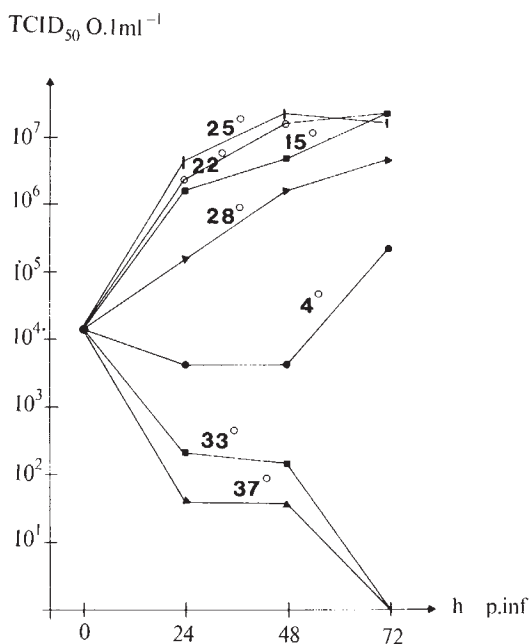


Fig. 1 Replication of isolate 10/3 in the FHM cell line at different incubation temperatures.

These negative results point more towards a viral aetiology and this is supported by a number of authors⁴⁻⁷ who successfully transmitted the disease with ultrafiltrates from brain and swim bladder suspensions prepared from carp with clinical symptoms of aerocystitis. A virus could not be demonstrated in these experiments, however, and inoculations of infectious organ material into cell cultures of carp origin were not successful⁸. This communication describes the aetiology of the disease as caused by a rhabdovirus.

During summer 1971 and spring 1972, serious outbreaks of swim bladder inflammation were observed in Germany. Brains and swim bladders were collected from diseased carp with typical symptoms of the acute form of the disease, ground in a mortar and diluted 1:10 in Earle's BSS. After centrifugation at 2,000g for 20 min the supernatant was passed through a 450 nm membrane filter and inoculated into primary cell cultures grown from the gonads and swim bladders of carp⁹ and into cultures of the cell line FHM¹⁰. Four tube cultures for each cell type were inoculated with 0.5 ml samples of the supernatant from the infected carp and, after an adsorption period of 1 h at 4°C, medium was added consisting of Eagle's minimal essential medium with 5% foetal calf serum and supplemented with 100 µg ml⁻¹ streptomycin, 100 µg ml⁻¹ penicillin, 50 µg ml⁻¹ neomycin, 50 µg ml⁻¹ polymyxin and 25 µg ml⁻¹ of the fungistat amphotericin. The cell cultures were incubated at 20–22°C for 7–14 d along with controls inoculated with uninfected cell culture material and checked daily for cytopathic effects (CPE).

A cytopathogenic agent was isolated from the materials in all cell cultures used. The CPE consisted of granulation and rounding up of cells followed by lysis. In haematoxylin-eosin stained preparations a distinct margination of chromatin was observed in the nucleus. In FHM cell cultures, lysis was complete about 72–96 h after infection. With increasing passage numbers in these cultures the isolate destroyed the cells more rapidly.

Infectivity titres recorded were between 10^{6.5} and 10^{7.5} tissue culture infective dose (TCID)₅₀/0.1 ml. Optimal virus replication was observed at temperatures between 15 and 28°C. The representative isolate 10/3 did not multiply at either 33 or 37°C, and showed only slow growth at 4°C (Fig. 1).

The disease could be reproduced by intraperitoneal inoculation of the eleventh FHM cell culture passage of isolate 10/3 into clinically healthy carp which weighed about 200 g. Each

fish was infected with 1 × 10⁶ TCID₅₀ of virus. Control groups were inoculated with uninfected cell culture material. The groups were checked daily for clinical signs, external lesions and mortality. Dead carp were investigated for histopathological changes and virus content of the different organs. In the infected group, clinical signs first appeared 3 d postinoculation. Apathy, swimming on one side, swelling of the abdomen and anus, and sometimes petechiae on the skin were observed. First deaths occurred 4 d after infection.

All experimentally infected carp died 4–8 d after infection. Post mortem changes were similar to those seen in naturally recorded acute swim bladder inflammation and were mainly dilatation of blood vessels and petechiae in the swim bladder wall and heart muscle, engorged brain vessels and ascitic fluid in the peritoneal cavity. Control fish remained healthy.

Virus was recovered from all organs of experimentally infected carp. The organs contained virus infectivity titres of between 10^{3.5} (brain) and 10^{5.5} (liver) TCID₅₀ g⁻¹. Organ pools from control fish inoculated in the same manner into FHM cell cultures proved negative in all virus isolation attempts. Chemical and physical characterization of the isolate 10/3 revealed particles with a buoyant density in caesium chloride gradients of 1.195 and 1.200 g cm⁻³, which is in the close range of rhabdoviruses¹¹ (Fig. 2). The gradient fractions containing highest infectivity showed numerous bullet-shaped particles in the electron microscope after staining with phosphotungstic acid (PTA), measuring 90–140 nm in length and about 70 nm in diameter (Fig. 3).

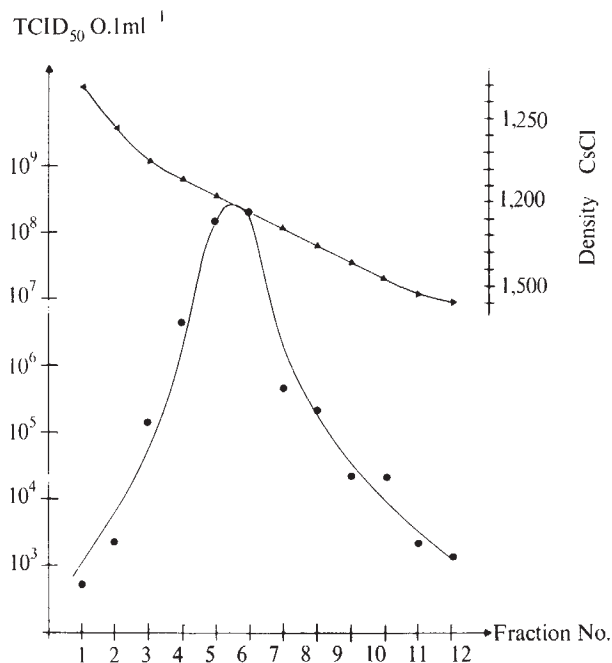


Fig. 2 Buoyant density of the 10/3 isolate in caesium chloride density gradients.

This morphology, together with the observation of an envelope bearing projections, resembles the structure of rhabdoviruses. The classification of isolate 10/3 as a rhabdovirus is also justified by the lack of inhibition of virus multiplication in the presence of a DNA inhibitor (10⁻⁴ M bromodeoxyuridine, Serva, Heidelberg), by its lipid solvent lability (chloroform), pH lability (60 min at pH 3), and heat lability (56°C, 30 min).

The 10/3 isolate from carp can be differentiated from the salmonid rhabdoviruses of haemorrhagic septicaemia (VHS)¹², infectious haematopoietic necrosis (IHN)¹³ and the agent of red disease in pike (*Esox lucius* L.)¹⁴ by its growth at different temperature optima (20–22°C compared with 10–15°C). The

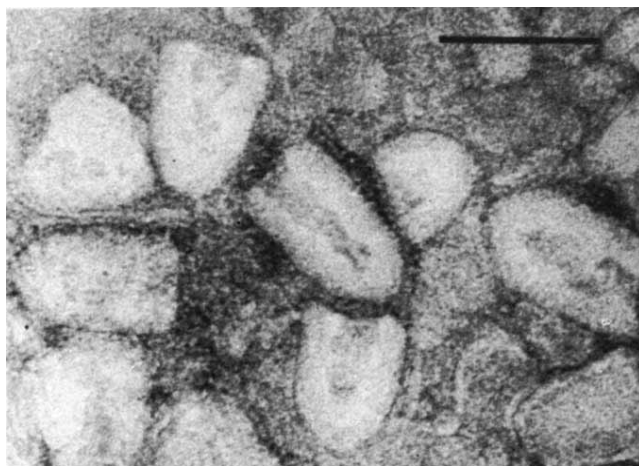


Fig. 3 Typical bullet-shaped rhabdovirus particles in the electron microscope, representing the virus isolate 10/3, PTA 2%, pH 6.7. (Bar equals 100 nm.)

optimal replication temperature is, however, identical to that reported for the agent of Spring Viraemia of Carp (SVC), another carp rhabdovirus, which has been identified as the causative agent of the acute form of Infectious Dropsy^{15,16}.

Up to now there has been no indication that Swim Bladder inflammation (aerocystitis) and Infectious Dropsy of carp may have a common aetiology. Whether these viruses are antigenically identical or related to each other, or whether the isolate 10/3 is related to other rhabdoviruses, remains to be shown in future investigations.

The isolation of yet another virus which is responsible for a disease emphasizes the importance of viruses in infectious diseases of pond fish.

This work was supported by the Deutsche Forschungsgemeinschaft.

P. A. BACHMANN

*Institute of Microbiology and Infectious Diseases of Animals,
University of München,
Veterinärstr. 13, 8000 München 22*

W. AHNE

*Institute of Zoology and Parasitology,
University of München,
Kaulbachstr. 37, 8000 München 22*

Received March 6, 1973.

¹ Kanaev, A. J., Lobuncov, K. A., and Naumova, A. M., *Ryb. Khoz.*, **43**, 16 (1967).

² Schäperclaus, W., in *Handbuch der Virusinfektionen bei Tieren*, **2**, 1067 (VEB Gustav Fischer, Jena, 1969).

³ Kocylowski, B., Antychowicz, J., and Zelazny, J., *Riv. Ital. Piscicult. Ittiopatol.*, **5**, 59 (1970).

⁴ Markiewicz, F., *Medycyna Wet.*, **22**, 16 (1966).

⁵ Kudenkova, R. A., *Symposium on Parasites and Diseases of Fish and Aqueous Invertebrates* (in Russian), **17** (Moscow, 1966).

⁶ Kudenkova, R. A., *Izv. Gos-Niorch*, **69**, 67 (1969).

⁷ Arsanica, N. M., *Izv. Gos-Niorch*, **69**, 5 (1969).

⁸ Rudikov, N. J., *Veterinarija*, **32**, 66 (Moscow, 1966).

⁹ Ahne, W., thesis (University of Munich, 1973).

¹⁰ Gravel, M., and Malsberger, R. G., *Ann. NY Acad. Sci.*, **126**, 555 (1965).

¹¹ Wildy, P., *Monog. Virol.*, **5**, 51 (Karger, Basel, 1971).

¹² Zwillenberg, L. O., Jensen, M. H., and Zwillenberg, H. L., *Arch. Ges. Virusforsch.*, **17**, 1 (1965).

¹³ Amend, D. F., and Chambers, V. C., *J. Fish. Res. Bd. Canad.*, **27**, 1285 (1970).

¹⁴ Kinkelin, P. de, Bootsma, R., and Galimard, B., *Nature*, **241**, 465 (1973).

¹⁵ Fijan, N. N., Petrinc, Z., Sulimanovic, D., and Zwillenberg, L. O., *Vet. Arh.*, **41**, 125 (Zagreb, 1971).

¹⁶ Fijan, N. N., *Symp. zool. Soc. Lond.*, **30**, 39 (1972).

Nitrosamines in Cigarette Smoke Condensate

THE toxicity of many *N*-nitrosamines has led to wide interest in their occurrence in foods such as cured meat products and smoked fish.

Rhoades and Johnson¹ have reported the detection of low levels of dimethylnitrosamine in the smoke condensate from several types of cigarette. Using gas-liquid chromatography (GLC) with a specific detector², they measured the peak with the same retention time as dimethylnitrosamine and, in a separate off-line experiment, confirmed the presence of this compound by high-resolution mass spectrometry.

In a parallel study we have analysed eight steam-volatile nitrosamines in the smoke condensate from a variety of commercial and experimental cigarettes (Table 1), using on-line GLC and high-resolution mass spectrometry³. The six experimental cigarettes, supplied by Dr T. C. Tso of the US Department of Agriculture, were made of tobacco produced especially for nitrosamine studies by elevating the rate of nitrogen fertilization. The high rate used for this experimental tobacco was three to four times that of the regular nitrogen rate used for normal tobacco production.

One hundred cigarettes were smoked and the condensate collected in a cold trap (-78°C). The methanolic condensate extract was steam distilled first from 10% aqueous potassium carbonate solution, then from saturated aqueous sodium chloride solution at pH 4. The distillate was extracted with methylene dichloride, the solvent reduced to a known volume and the sample maintained at -78°C until analysed. Each sample was analysed by GLC (10% Ucon Oil 58 HB 28X on 'Celite') and on-line high-resolution mass spectrometry (MS902). As each nitrosamine eluted from the column the appropriate accurate mass region of the molecular ion was scanned.

Table 1 Cigarette Types

Cigarette description	Tobacco blend
Commercial cigarettes	
Flue-cured	UK blend of flue-cured tobaccos
French	French blend containing predominantly air-cured tobacco
German	German blend containing flue-cured, oriental and Burley tobacco
Swiss	Swiss medium air-cured blend containing greater than 50% Maryland tobacco
Cigar	Small UK cigar
Experimental cigarettes	
Catterton (l)	Maryland tobacco grown on low nitrogen soil
Catterton (h)	Maryland tobacco grown on high nitrogen soil
Robinson (l)	Maryland tobacco grown on low nitrogen soil
Robinson (h)	Maryland tobacco grown on high nitrogen soil
Burley (l)	Burley tobacco grown on low nitrogen soil
Burley (h)	Burley tobacco grown on high nitrogen soil

Serial dilution of all of the nitrosamines both as individual compounds and as a mixture gave straight line calibration curves for the abundance of the molecular ion against concentration. An appropriate standard solution of the mixture of nitrosamines was run before and after each smoke condensate analysis. Smoke solutions spiked with known amounts of the nitrosamine mixture were also regularly run as a further check on the analysis.

One set of samples was analysed as soon as possible after smoking and again after 3 weeks. No changes in the analytical values were found, showing the long-term stability of these solutions.

The analytical values are given in Table 2 along with the total volatile base content of the tobacco and the tobacco

Table 2 Nitrosamines in Smoke Condensate Extracts

Tobacco type or blend	Tobacco analyses		Delivery of nitrosamine in ng per cigarette								Dimethyl (from ref. 1)
	Total volatile bases, % N	Total nitrate, % NH ₃	Dimethyl-	Ethyl-methyl-	Diethyl-	n-Butyl-methyl-	Di-n-propyl-	N-Nitroso-pyrrolidine	N-Nitroso-piperidine	Di-n-butyl-	
Flue-cured	0.25	0.06	5	nd	nd	nd	nd	6	nd	nd	—
French	0.56	0.27	143	12	6	nd	nd	110	nd	nd	—
German	0.30	0.19	10	1	1	nd	nd	26	1	3	—
Swiss	0.42	0.24	17	2	1	nd	1	16	3	3	—
Cigar	0.64	0.31	160	40	28	nd	nd	42	nd	nd	—
Catterton (l)	0.38	0.11	19	nd	nd	nd	nd	5	nd	nd	5
Catterton (h)	0.49	0.54	120	9	2	nd	nd	55	7	nd	60
Robinson (l)	0.22	0.07	9	nd	nd	nd	nd	6	nd	nd	0
Robinson (h)	0.46	0.53	110	4	2	nd	nd	68	5	nd	27
Burley (l)	0.44	0.13	11	nd	nd	nd	nd	1	nd	nd	3
Burley (h)	0.85	1.02	180	13	4	nd	nd	76	9	nd	140
Detection limit	0.02	0.05	0.2	0.3	0.4	1.0	0.5	0.5	0.4	1.0	

nd, Not detected.

nitrate analysis. The earlier results¹ on dimethylnitrosamine are also included.

From these results it seems that the levels of nitrosamines found are dependent on the level of total volatile bases in the tobacco and on the tobacco nitrate content.

ANDREW McCORMICK
MICHAEL J. NICHOLSON

*Physico-Chemical Measurements Unit,
Atomic Weapons Research Establishment,
Aldermaston, Reading RG7 4PR*

MICHAEL A. BAYLIS
JOHN G. UNDERWOOD

*Group Research and Development Centre,
British-American Tobacco Co. Ltd,
Regent's Park Road,
Southampton SO9 1PE*

Received March 21, 1973.

¹ Rhoades, J. W., and Johnson, D. E., *Nature*, **236**, 307 (1972).

² Rhoades, J. W., and Johnson, D. E., *J. Chromatog. Sci.*, **8**, 616 (1970).

³ Bryce, T. A., and Telling, G. M., *J. Agric. Food Chem.*, **20**, 910 (1972).

Model which Generates Red Tides

DINOFLAGELLATES and *Trichodesmium* are the algae most commonly responsible for the discolorations of the sea known as red tides. These blooms are rarely observed until their development is complete, so that the events which lead to red tides remain enigmatic. Dinoflagellates are distinguished by their relatively large size compared with other flagellated algae, and by their greater swimming speeds. Many of them produce substances which when released into the water are toxic to animals. Usually any toxin released is probably either destroyed or diffused as rapidly as it is produced, and the species remains harmless, but in special circumstances, not yet adequately identified, the dinoflagellate and its toxin become very abundant and a red tide occurs, which, if prolonged, may

be followed by mass mortality of fish and other organisms. *Trichodesmium* is also motile in the sense that it can produce gas vacuoles which cause it to rise in the water column.

The literature, summarized by Brongersma-Sanders¹ and Rounsefell and Nelson², reveals certain conditions which frequently precede outbreaks of red tide. Commonly reported conditions are heavy rainfall, calm sunny weather, an influx of estuarine waters and meeting of dissimilar water masses. A likely result of any one of these is an increase in the vertical stability, either by an increase in the density gradient or by a decrease in the wind-induced vertical coefficient of eddy diffusion. Both result in a reduction in vertical mixing rates. Such conditions allow motile organisms like dinoflagellates to concentrate more easily in preferred layers, where light is optimal for reproduction. As they are not then distributed throughout the photic zone, their average reproductive rate will increase. With calm sunny weather, the surface albedo will be low, the level of illumination maximal and this effect may be amplified.

In normal conditions, phytoplankton is distributed throughout the mixed layer, and subjected to grazing pressure by the herbivorous zooplankton. As the bulk of the zooplankton is able to adjust its level in the water column under normal mixing conditions, there is no reason to suppose that its behaviour is altered by an increase in stability, and it is reasonable to assume that the distribution of grazing pressure in the water column remains unchanged by such an increase. The migration of a phytoplankton species to a restricted part of the water column will therefore result in its release from most of the grazing pressure to which it is normally subject and, if the total grazing pressure remains constant, the non-migratory species will be subjected to more intense mortality. Thus an increase in stability, resulting in the layering of a motile species, will simultaneously release that species from the constraints of sub-optimal illumination and grazing pressure, and could lead to its very rapid dominance. Unrestricted growth of this kind is found in laboratory unialgal cultures of limited volume. Such growth cannot continue exponentially for an indefinite period, because either nutrients are depleted or the algae become so dense that light once again becomes limiting. In cultures grown in these conditions, colour changes occur, from green to yellow, amber, brown or red. The same colours usually characterize red tide outbreaks. The rarely reported milky-white effect of *Trichodesmium* blooms is perhaps an optical effect dependent on their gas vacuoles. Continuation of these conditions leads to a cessation of reproductive activity and to cell death. The loss of nutrients

would be exacerbated by the absence of zooplankton from the red tide layer, as nutrients are normally rapidly regenerated by grazing. In the sea, one would expect this final phase to result first in the release of toxic substances and then putrefaction and oxygen depletion possibly resulting in poisoning and death of other organisms. The same sequence could be brought about by light limitation, with nutrients remaining plentiful. The chain of events suggested here is summarized in Fig. 1.

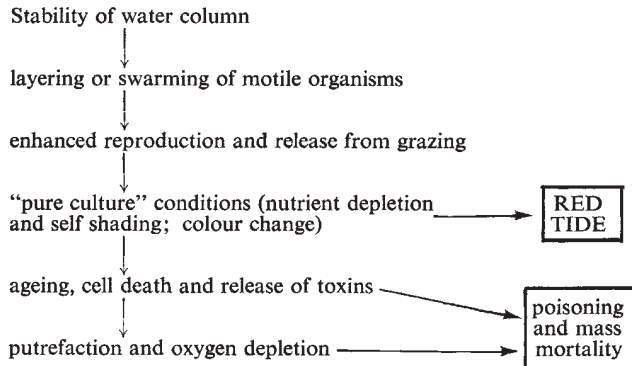


Fig. 1 Suggested chain of events leading to an outbreak of red tide.

It is perhaps significant that the few authors who have observed the termination of red tide conditions have suspected that an increase in wind strength was responsible. This would follow from the hypothesis presented. The first four links in Fig. 1 can all be reversed by the increase in vertical mixing which an increase in wind would bring about. Occasionally it has been noted that red tides change to green, a possible reversal of the fourth step.

If we assume that grazing is constant, then the changes in algal numbers can be expressed as follows:

$$A_{pt} = A_{p0} \exp[(R_p - G_{pt})t] \quad (1)$$

$$A_{qt} = A_{q0} \exp[(R_q - G_{qt})t] \quad (2)$$

and

$$G_t = G_{pt} + G_{qt} = C, \text{ a constant} \quad (3)$$

where A_{p0} and A_{q0} are the initial numbers of non-motile and motile algae, A_{pt} and A_{qt} are the numbers at time t , R_p and R_q are the respective reproductive rates, and G_p and G_q are the grazing rates.

The reproductive rate, r , at any given depth, z , can be expressed by an equation of Steele³ of the form

$$r = r_m(I_z/I_m) \exp(1 - I_z/I_m) \quad (4)$$

where I_z is the radiation reaching depth z , I_m is the radiation level at which maximum reproduction occurs, and r_m is the maximum reproductive rate. We also know that

$$I_z = I_0 \exp(-kz) \quad (5)$$

where I_0 is the radiation reaching the surface and k is the extinction coefficient. Combining (4) and (5), we obtain

$$r = r_m(I_0/I_m) \exp[1 - k z - (I_0/I_m) \exp(-kz)]$$

The mean reproductive rate, R , over a depth $z = d$ is obtained by integration,

$$\begin{aligned} R &= (r_m I_0 / d I_m) \int_0^d \exp[1 - k z - (I_0/I_m) \exp(-kz)] dz \\ &= (r_m \exp(1/dk)) [\exp(-(I_0/I_m) \exp(-kd)) - \exp(-I_0/I_m)] \end{aligned}$$

which can be factorized to

$$R = (2r_m \exp(1/dk)) \exp(-1/2(\eta + \theta) I_0) \sin h(1/2(\theta - \eta) I_0) \quad (6)$$

where

$$\eta = (I_0/I_m) \exp(-kd) \text{ and } \theta = I_0/I_m$$

When the stability of the water column is such that the motile algae are able to congregate at a particular depth, it may be assumed that they will choose a depth at which the reproductive rate is maximal. This will be when $I_m = I_0 \exp(-kz)$ and will generally be at or very close to the surface. Thus

$$Z = 1/k \log_e(I_0/I_m) \text{ when } I_0 \geq I_m$$

and

$$z = 0 \text{ when } I_0 \leq I_m$$

Consequently

$$R = r_m \text{ when } I_0 \geq I_m$$

and

$$R = r_m(I_0/I_m) \exp(1 - I_0/I_m) \text{ when } I_0 \leq I_m$$

The rates at which R approaches these values will be determined by the swimming speed in the case of dinoflagellates, and by the rate of vertical eddy diffusion. Now, if the number of grazers remains constant, an increase in the abundance of a particular species of algae will result in a fall in the grazing coefficient of that species. If, for simplicity, we assume a time lag δt in this effect, we may say that

$$G_{q(t+\delta t)} = \gamma(A_q)^{-1} \quad (7)$$

and from (3)

$$G_{p(t+\delta t)} = C - \gamma(A_q)^{-1} \quad (8)$$

Substituting (7) and (8) into (1) and (2), and letting δt be one time step, an explicit relationship is obtained:

$$A_{p(t+\delta t)} = A_{pt} \exp[(R_p - C + \gamma(A_{qt})^{-1})\delta t] \quad (9)$$

$$A_{q(t+\delta t)} = A_{qt} \exp[(R_q - \gamma(A_{qt})^{-1})\delta t] \quad (10)$$

The condition expressed in equation (7) will only obtain when numbers are relatively high, as in the quasi-steady state which exists in tropical and subtropical plankton communities, and in temperate regions towards the end of the summer. This effect is in addition to the reduction in grazing consequent on layering, and will be most marked in that layer.

A position was chosen at 28° N, with ocean water type III⁴, to correspond to the situation off the coast of Florida. The elevation of the Sun at hourly intervals was obtained from nautical tables for late November to early December, and from these the solar radiation at the surface was obtained⁵ assuming a clear sky. The extinction coefficient was taken as $K = 0.142$ (ref. 4), and I_m as 90 langley d⁻¹ (ref. 3). Using equation (6), with a depth of 50 m, a value of $r_m = 0.072$ h⁻¹ gave an average hourly $R = 0.0125$ over a day. Using these values, with $C = 0.025$ ($= 2R$), and setting γ at different levels, the results shown in Fig. 2 were obtained. There is an exponential increase in the number of motile algae (A_q), and a general fall in the number of non-motile forms (A_p), though they may increase slightly at first, when γ is high. The slopes depend on γ , the density-dependent grazing coefficient. The slopes will also depend on the rate of change of the vertical coefficient of eddy diffusion, which is not made explicit in the model, and it is possible that this will be the most important factor. Given the instantaneous change assumed here, from well mixed to very stable conditions, and a low value for γ , it is obvious that very high cell concentrations can develop rapidly.

Existing evidence suggests that the toxic substances found in dinoflagellates are only released when the cells become senile or die⁶. If, however, a poison were secreted continuously

as a protection against grazing, then the concentration of toxin, T , could be expressed as $T = \lambda A_q - f(w)$, where λ is a constant, and w is the vertical component of eddy diffusion. Then when $w \rightarrow 0$, $T = \lambda A_q(t - \delta t)$, and, if the grazing pressure on the toxic species were inversely proportional to the concentration of toxin, $G_{qt} = \lambda [A_q(t - \delta t)]^{-1}$, which has the same form as equation (8). Production of poison would therefore have the same effect as γ , the grazing coefficient.

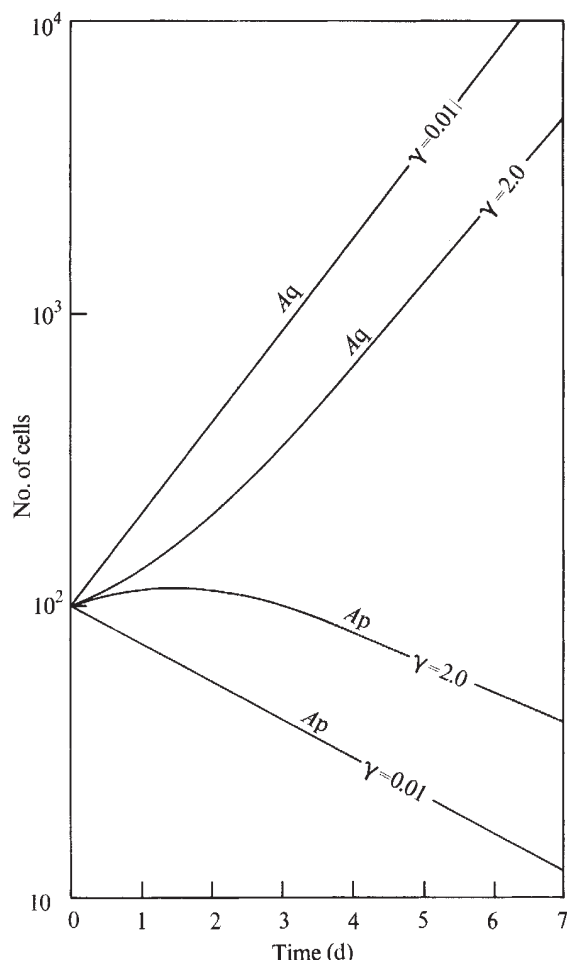


Fig. 2 Increase of motile algae (A_q) and decrease of non-motile algae (A_p) for different values of the grazing coefficient, γ , against time.

Most views on the causes of red tide fall into two groups, which may be called nutrient theories and hydrographic theories. The former seek to explain outbreaks of red tide on the basis of nutrient enrichment, either through run-off from the land, through upwelling, through the intermittent release of phosphate from phosphatic outcrops on the sea-floor, or through the discharge of nutrient-rich sewage. None of these theories is adequate to explain the occurrence of red tides in deep water far from the land. More subtle nutrient theories suggest that the water must be conditioned in some way by other organisms. This idea generally invokes heterotrophic metabolic requirements on the part of the red tide species. Cultural evidence⁷ indicates that for *Gymnodinium breve*, at least, no such requirements exist. More ingenious is the idea that certain growth factors normally occur separately in different water masses, and that blooms cannot take place unless these are mixed together. This idea is also untenable for the same reasons. The suggestion made here, however, that the red coloration and eventual limitation of these blooms

is brought about by nutrient depletion, indicates that any nutrient enrichment, from whatever source, could affect the detailed course of events during an outbreak, and, in particular, influence the cell concentrations achieved.

Hydrographic theories have dealt mainly with the horizontal distribution of red tide patches. The process of convergence has frequently been held responsible for the concentration of red tide organisms, and other plankton, into streaks and patches. Whether mechanical concentration alone is sufficient to account for the high cell densities observed is not known but seems unlikely. Slobodkin⁸ suggests that intense reproduction may occur in isolated cells of water situated along a convergence. Clearly, any kind of convective process would require the organisms to be either motile or negatively buoyant for them to remain near the surface of the convergence. Less attention has been paid to vertical water movements, but a few observers⁹ have suggested that dinoflagellates become dominant when vertical mixing is at a minimum. Vertical stability is, of course, central to the hypothesis developed here, and can best be expressed by the Richardson number, defined as

$$R_i = g/p(\delta p/\delta z)[1/(\delta u/\delta z)^2]$$

where g is the acceleration due to gravity, p is the mean density of the water column, and u is the water velocity. Estimates of vertical stability therefore require current measurements to be made, and these have not yet become part of routine oceanographic survey work.

There are no published data which allow us to test this model's prediction that, given the presence of the red tide organism, it will become more abundant with increasing vertical stability. Presumably physical processes are of relatively more importance in the production of red tide outbreaks by non-autotrophic organisms, such as *Noctiluca* and ciliate protozoans. The hypothesis outlined here might be examined experimentally using the vertical tank described by Strickland *et al.*¹⁰.

T. WYATT
J. HORWOOD

MAFF, Fisheries Laboratory,
Lowestoft, Suffolk

Received March 26; revised May 25, 1973.

- ¹ Brongersma-Sanders, M., *Mem. geol. Soc. Am.*, **67**, 941 (1957).
- ² Rounsefell, G. A., and Nelson, W. R., *Spec. scient. Rep. U.S. Fish. Wildl. Serv., Fish.*, **535** (1966).
- ³ Steele, J. H., *Mem. Ist. ital. Idrobiol.*, **18**, 383 (1965).
- ⁴ Jerlov, N. G., *Rep. Swed. deep Sea Exped.*, **3**, 1 (1951).
- ⁵ Lumb, F. E., *Q. Jl R. met. Soc.*, **90**, 43 (1964).
- ⁶ Prakash, A., Medcof, J. C., and Tennant, A. D., *Bull. Fish. Res. Bd Can.*, **177** (1971).
- ⁷ Aldrich, D. V., *Science*, N.Y., **137**, 988 (1962).
- ⁸ Slobodkin, L. B., *J. mar. Res.*, **12**, 148 (1953).
- ⁹ Sykes, J. E., *Spec. scient. Rep. U.S. Fish. Wildl. Serv., Fish.*, **521**, 9 (1965).
- ¹⁰ Strickland, J. D. H., Holm-Hansen, O., Eppley, R. W., and Linn, R. J., *Limnol. Oceanogr.*, **14**, 23 (1969).

Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Archaeological View

An Archeological Perspective. By Lewis R. Binford. Pp. xii+464. (Seminar: New York and London, June 1972.) \$11.95.

LEWIS BINFORD is unquestionably the most influential figure in archaeology today. The principal originator in America of the "new archaeology", with its methodological concern to set the structure and logic of archaeology on a more rigorously scientific footing, his ideas have stimulated much of the most interesting recent work on both sides of the Atlantic.

An Archeological Perspective is a selection of papers, many of them previously published in inaccessible periodicals, introduced and linked by connecting passages which are personal and autobiographical in nature. The papers together constitute the most thoughtful and critical discussion yet written of the problems of the interpretation of archaeological data. They are philosophical, dense and sometimes rather difficult to read. They form, however, as conveniently presented here, an indispensable basis for anyone seriously concerned with the scope and limitations of prehistoric archaeology. Taken together they rank, in my view, as the most important conceptual advance since Gordon Childe was formulating his own methodological basis nearly fifty years ago.

Some of these papers have already taken their place among the classics of archaeology. "A consideration of archaeological research design" (1964), for example, was the first serious discussion of the difficult problem of sampling in archaeological data collection. "Archaeological systematics and the study of culture process" (1965) introduced the systems approach to archaeology, and has stimulated much further work. "Post-Pleistocene adaptations" (1968) remains the clearest embodiment of the ideal that archaeological explanations should be very general, preferably of lawlike form, and offers the most intelligent and provoking general discussion available of the origins of farming.

Much of Binford's recent work has been directed towards the palaeolithic period, and "Model building, paradigms and the current state of palaeolithic research" is a new review of this field. It is a pity, however, that his innovative paper (with S. R. Binford), "A preliminary analysis of functional variability in the Mousterian of Levallois facies" (*American Anthropologist*, 68, 238; 1966) is not included: as the first

application of factor analysis to archaeological data it is of real historical and methodological interest.

Among the papers relating to later periods, "Mortuary practices, their study and potential" (1971) will be useful to any archaeologist thinking seriously about funerary remains. And the extract from the Hatchery West excavation report, indeed the report as a whole, should be read by any field-worker grappling with the problems of rescue excavation.

None of these makes easy reading. In complete contrast, the introductory passages are autobiographical in content and colourful, frank and uninhibited in style—racy is the only word. If you wish to know what Binford thinks of Professor R. J. Braidwood for example (no love lost), it is unambiguously spelt out here. The historian of the future may value this—workers today will find it as entertaining, and perhaps as revealing, as Watson's *Double Helix*.

The book as a whole, like its author, is a lively combination of a tough mind undertaking highly original abstract analyses and a colourfully forceful personality. It offers a real insight into the arduous task, still far from complete, of elevating archaeological reasoning from the level of intuition to that of a rational discipline.

COLIN RENFREW

Psychosomatics

Physiology, Emotion and Psychosomatic Illness. Pp. viii+421. (Elsevier/Excerpta Medica / North - Holland: Amsterdam, London and New York, 1973.) Dfl. 53; \$16.50.

THIS is the book of a symposium held in April 1972. It is well produced, and has been published without excessive delay. There are seventeen papers. Each, with one exception, is followed by a discussion. The discussions are documented, and they have been edited to be coherent and concise; but plenty of hard-hitting comments remain.

The paper which lacks a discussion is the first, by R. A. Hinde, on the "Concepts of Emotion". This is not surprising: as Wittgenstein said, in psychology we have experimental methods and conceptual confusion, and problems and methods pass one another by. "Emotion", with all its meanings and use in diverse contexts, perhaps always does refer to "an interrelated set of questions"; but this does not ensure effective communication between people whose criteria of validity differ as much as those of psychiatrists and experimental psychologists.

After Hinde's chapter there are three on psychoanalytical themes: those by J. C. Nemiah and by G. L. Engel with A. H. Schmale give some readable case histories; one, by J. Sandler, on affect, illustrates some of the theoretical difficulties of psychological medicine and its perhaps inevitable separation from experimental science. At the end is a further group of psychiatric papers. M. Lader questions the value of the term "psychosomatic", but gives a general review of the "psychophysiological" approach to medicine. G. Tibblin and colleagues describe observations of great interest on cardiovascular function in relation to heart disease and to emotional states. P. Storey presents a series of cases of subarachnoid haemorrhage. J. Crinker and J. Hirsch provide an excellent account of the metabolic and behavioural correlates of obesity.

In the middle are eight chapters on experimental studies, mostly of laboratory mammals. J. A. Gray and E. Fonberg each contribute a chapter on the limbic system. B. E. Ginsburg writes on the unexpected topics of domestication and taming.

Several contributions are on cardio-respiratory function. That by M. A. Hofer concerns the heart function of infant mammals during separation from the mother. This chapter adds an ontogenetic dimension to the experimental studies but, as usual, theoretical links were hard to find. A. Zanchetti and his colleagues present another series of elegant experimental findings, on the cardiovascular changes of a cat faced with another cat, a dog or a mouse.

J. P. Henry and others describe some original observations concerning the pathogenic effects on mice of social isolation. In their experiments special attention is given to the catecholamines, blood pressure and social interactions. J. M. Weiss gives evidence that gastric histopathology in experimental animals is related to the amount of "coping" behaviour the animal performs, and the information it receives as a result of this behaviour. Weiss discusses this in terms of "psychological variables". (Under social pressure, he later defined "psychological" in terms of stressful external stimuli.) S. Levine and others, in a chapter entitled "Expectancy and the Pituitary-Adrenal System", show how the method of "operant conditioning" in a Skinner box can be used to investigate physiological changes that accompany habit formation.

In spite of the ability of the authors, this collection certainly confirms Wittgenstein's criticism. Nevertheless, I found it immensely interesting. Perhaps the participants would have gained more

had they spent almost all the time in discussion; but this would not have generated a book. S. A. BARNETT

Electrical Interfaces

Interfacial Electrochemistry. By R. K. Ottewill, J. Lyklema and R. Parsons. (Reprinted from the *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry*. Volume 37.) Pp. 426. (Elsevier: Amsterdam, London and New York, 1972.) Dfl. 100; \$31.25.

The Electrical Double Layer. By M. J. Spaarnay. (*The International Encyclopedia of Physical Chemistry and Chemical Physics. Topic 14: Properties of Interfaces.* Volume 4.) Pp. xii+415. (Pergamon: Oxford and New York, December 1972.) £6.50.

THE study of interfaces is necessarily a demanding process. The number of phases and the number of components are often large and the simplest formulation becomes complex. The thickness of the interface may be vanishingly small and is likely to be bounded by zones of considerable anisotropy. Moreover, the interface invariably concentrates many otherwise minor impurities and its experimental investigation is no less difficult than its theoretical formulation. Indeed, from both points of view the physics and chemistry of interfaces presently lack much of the elegance, simplicity and certainty of, say, spectroscopy and if it is true that most searchers prefer to look where the light is, then it is not surprising to note that the study of interfaces lags considerably in its range of specific instrumentation and in its rate of progress.

This is so in spite of the importance of interfacial studies to almost all systems of practical significance including, for example, those involving reacting solids, semiconductors, enzymes and other heterogeneous catalysts, nerve cells, as well as all foodstuffs both growing and eaten. It is therefore fortunate that there remain groups of researchers actively investigating the fundamentals of interfacial phenomena and there are now signs of large strides about to be taken as the result of applying spectroscopic and other optical techniques to these systems. However, our present understanding rests firmly on thermodynamic methods and it is interesting to note the large contribution made to this branch of physical chemistry by the Dutch schools. It may be fanciful to suppose that the analytical discipline appeals to the Dutch temperament, although it is certain that their broad and extended academic education fits them well for these interdisciplinary researches. Perhaps one should also not overlook the peculiarly close relation between Dutch universities and Dutch industry, especially Philips of Eindhoven. Whatever the cause, it is certain

that much of our present understanding of interfaces and particularly those of the solid-solution interface stems from Dutch laboratories and in the beginning from the guiding hand of Professor Overbeek. His delineation of colloidal behaviour was a milestone in the progress of this ancient but hitherto qualitative science and one of the books under consideration here is a collection of some thirty-eight papers on interfacial phenomena by students and colleagues of Overbeek, most of them, but by no means all, still working in Holland. This impressive set of papers was presented to Overbeek to mark the twenty-fifth anniversary of his appointment as professor at Utrecht and they deal with topics close to his interests, i.e. with double layer effects, electrokinetic phenomena, emulsions, membranes and some biological systems. It is a most useful summary of the present situation and deserves to be widely read.

The second book reviewed here is by one of Overbeek's most distinguished students, M. J. Spaarnay, with whom in 1957 he undertook the direct measurement of Van der Waals' forces. Spaarnay is well known for his thoughtful and solid contributions to the field and this is reflected in his book. It is a thorough and classical treatment of the electrical double layer (1) at the mercury-aqueous interface, (2) at the silver iodide-aqueous interface, (3) in the presence of surfactants, and (4) at semiconductor surfaces. The first of these includes a particularly valuable account of the inner part of the double layer and the second deals with a topic invariably overlooked in electrochemical texts. There is an introductory chapter of some 60 pages describing the thermodynamics of interphases and there is an epilogue containing useful generalizations on aspects still to be resolved. Apart from one or two ambiguities the text is clear and well set out. It is almost everywhere an authoritative account and each chapter is impressively supported by an extensive and recent set of references. There is only occasional reference to experimental procedures and to actual results because the stated aim is to set out the foundations of the subject and this is done here as well as anywhere. As is often the case rigour is achieved at the expense of easy understanding and this is not a book for beginners.

It also remains throughout a classical book. Notwithstanding the molecular dimensions of the interface and the importance of short range forces, the interface is firmly subjected to the concepts of time and space averaging, which ultimately exclude the important fine detail of molecular behaviour in the interface including the dynamic properties. The way out of these restrictions is experimentally through spectro-

scopic studies and theoretically through the advances in the statistical mechanics of liquids, especially those based on numerical methods. These ways forward are not evident in either of these books because they are as yet speculative and possibly far from success. Both volumes, however, describe well the present level of achievement and are strongly recommended to all those actively investigating interfacial phenomena. GRAHAM HILLS

Perils of the Embryo

Drugs and Fetal Development. Edited by Marcus A. Klingberg, Armand Abramovici and Juan Chemke. (Proceedings of and International Symposium on the Effect of Prolonged Drug Usage on Fetal Development, held at Kfar Saba, Israel, September 1971.) Pp. xiv+559. (Plenum: New York and London, 1972.) \$32.

THIS book is an edited account of the proceedings of an international symposium held in Israel in the autumn of 1971. It comprises some forty-four papers on a variety of topics relating to the central theme, some being reviews, some reports on original work, some essays on methodology. The reader is spared both the profundities and banalities of whatever discussion followed each communication, although presumably stimulation of discussion was the motive for bringing the participants together, and without it the material presented lacks coherence.

The symposium proper opens with an erudite historical introduction by Dr Warkany (the doyen of developmental pathologists) entitled "Congenital Malformations through the Ages". Subsequent contributions deal with both experimental and clinical observations, beginning with a review of reported adverse effects of drugs on the human foetus by Shirkey, who cites over 100 references, and continuing with a number of useful papers in which the significance and relevance of animal studies to possible effects on the human foetus are critically discussed. A further paper by Rennert goes into the mechanisms by which some agents affect protein synthesis in the embryo and is followed by interesting observations by Keeler on the teratogenic effect on grazing animals of certain poisonous plants and by the Lutzes on the effects of pesticides in inhibiting Müllerian suppression in bird embryos. Subsequent contributions include the inevitable description of Minamata disease by Murakami, an important paper by Laron on the foetal actions of growth hormone, and a disturbing study reported by Richards on possible effects on the foetus of salicylates taken during pregnancy. It is reassuring that Ravid and Toaff were not able to show any such effects from

the taking of antibiotics, albeit there is evidence in other papers that the tetracyclines, and chloramphenicol in particular, have demonstrable effects on mitochondria and molecular transcription. Another important paper is that by Choi *et al.* on congenital central nervous system malformations in Manitoba, which they find to be associated with low maternal calcium levels.

The book is completed by a useful list of participants and an inadequate index which is not a helpful guide to the contents.

Taken as a whole, this book is likely to be of value mainly to obstetricians, paediatricians and experimental pathologists interested in teratology for whom it provides a helpful set of references and a guide to work and workers in their field. Disappointingly it deals only superficially with the mode of action of teratogenic agents either from the point of view of pharmacology or embryology; and of course it is already partly out of date.

The price is on the steep side for what is essentially an ephemeral source of information, albeit it is well produced and surprisingly easy to read considering that many contributors are not using their own language. The publishers should change the "blurb" on the dust jacket which is almost totally misleading.

J. DAVIS

Muscle Sense

Mammalian Muscle Receptors and Their Central Actions. By Peter B. C. Matthews. Pp. x+630. (Edward Arnold: London, 1972.) £9.

It is a remarkable testimony to the complexity of the sensory receptors of muscle and to the difficulty of their experimental study that after some hundred years of research so much is known yet so much remains to be settled. The story of this research as told in Dr Peter Matthews's words forms an absorbing example of the way in which a biological science typically progresses. A most helpful feature of the book is that a historical path is marked through the maze of detail which would otherwise be so confusing. We see how a limited level of understanding was reached by micro-anatomical study, but that further advance had to await the new techniques of electrical recording in the 1930s. A period of rapid advance followed until by 1960 the understanding of muscle spindles had reached a stage consistent enough perhaps to satisfy the less enquiring mind and to justify calling it the "classical picture". In this, the spindle consisted of special muscle fibres bearing two types of sensory endings and a motor supply. Two new developments then took place to cause renewed interest, which is still maintained. The

intrafusal muscle fibres were shown to be of two kinds and the motor innervation was found to have unexpected complexities.

The whole subject is dealt with in a most scholarly manner and with the critical insight which we would expect from Dr Matthews, who has contributed such a large part of our original knowledge of the muscle receptors in recent years. There is a temptation in any subject as complex as this for the individual research worker to embark on a solitary exploration of some aspect which, while interesting in itself, is not on the direct route to the most rewarding objectives. There are, for example, the very interesting questions of the biophysics of the origin of the receptor potential and the initiation of nerve impulses in the spindle. There are the questions of the exact distribution of motor fibres to intrafusal muscle fibres and the origins of velocity sensitivity. These problems continue to engage the attention of many workers and Dr Matthews does them justice in appropriate chapters, but he is not sidetracked by them. Instead the development of the material is followed logically to the final chapters concerned with the place occupied by the muscle receptors in voluntary muscle control. These contain an admirable and fairminded presentation of a difficult subject, although there may be some disappointment that control engineering principles are not better presented. It is true that oversimplification is difficult to avoid in applying such principles in this area, but without them the ideas of servo control and servo assistance are really beyond intuitive grasp. Much argument has centred on attempts to demonstrate that voluntary movements may be driven through the fusimotor route. The view, as originally stated and since repeatedly implied, was that it is only by driving by this route that the advantages of a servo control are made available. The control engineer would not take this view, because if the only function of the fusimotor drive were to provide a "bias" on the spindle output as an excitatory input to the motor neurones, this could just as well be sent direct to the motor neurones. Of course, muscle spindles tend to be silenced by muscle shortening so that α - γ linkage may be desirable to keep spindles active during shortening to maintain feedback control. But this is already achieved in amphibians without a special fusimotor system. The implication therefore is that the mammalian fusimotor system must have other significance. Dr Matthews does arrive at virtually this conclusion, but he might have done so more directly from control engineering principles. Nevertheless, the author has done great service, to those seeking to use control

systems analysis methods, by his work on transfer function estimation by measuring the small amplitude vibration sensitivity of spindles. The sinusoidal testing method is described and the results which at first sight conflict with those of larger scale transient testing are explained, at least in part. Readers should be warned of a small error in the expression quoted for the response amplitude as a function of frequency.

This work is going to be of lasting value to research workers and students for many years. It expands and updates the author's widely quoted review of ten years ago and leads us to hope that perhaps ten years from now a further volume will appear with the answers to many of the problems which are formulated in such precise terms in this one.

A. TAYLOR

Explanation in Science

Causality and Scientific Explanation. By William A. Wallace. Volume 1. *Medieval and Early Classical Science.* Pp. xi+288. (The University of Michigan: Ann Arbor, November 1972.) \$12.

THIS is the first of a proposed two-volume work, the thrust of which is to demonstrate the continuing role which the notion of causality has played in discussions of scientific explanation from antiquity to the twentieth century. In developing his theme Professor Wallace attempts to combine the analytic approach of the philosopher of science with the factual one of the historian of science. His principal thesis is that causal explanations continued to have a more important function in science after the Middle Ages than is normally admitted. Before the second volume appears one cannot say how successful he is and, indeed, the burden of proof lies in showing that causal explanations continued to have importance in the development of post-Newtonian science.

This volume does show quite convincingly that causal explanations retained an important position in seventeenth-century scientific thought, for example in Galileo, Harvey and Newton. Once one penetrates a bit beneath the surface of these writers a strong motivation to put forth causal explanations is found to persist in spite of various denials.

Leaving aside the central thesis, it can be read as a connected treatment of scientific methodology from Aristotle to Newton. As such it is most useful, especially since it is based on a wide reading of little known primary sources and some of the best recent secondary literature. Several particular aspects of Wallace's work are noteworthy. First, it stresses in convincing fashion a continuity between mediaeval and

early modern science by pointing to specific connexions. Secondly, it gives a much fuller and more informed treatment of the fifteenth and sixteenth century developments than is usual. Thirdly, it rightly emphasizes the very deep ancient, mediaeval, and Renaissance influences at work on men like Galileo, Harvey, and Newton.

One can, however, disagree with the author on various points of detail and, indeed, on certain major issues. It seems to me that non-Aristotelian modes of scientific explanation and causality are not adequately dealt with. In fact, very little is said of the traditions of magic and pseudo-science, though it is becoming increasingly apparent that these had a more significant role in the development of "modern science" than had been realized. The book is challenging, however, and one hopes that both historians and philosophers of science read it, for, among other things, it provides a richer range of examples than is usually accounted for. It is scrupulously documented, has an excellent working bibliography, and is well indexed.

CHARLES B. SCHMITT

Plant Biosystematics

Taxonomy, Phytogeography and Evolution. Edited by D. H. Valentine. Pp. xi+431. (Academic: New York and London, October 1972.) £7.20.

THIS volume records the proceedings of a symposium held by the Linnean and Botanical Societies of the British Isles in association with the International Organization of Plant Biosystematists.

The papers included vary greatly in nature and approach: some deal with certain small groups (for example, *Epilobium*, *Alchemilla*, *Nothofagus*), while others discuss whole families (Combrataceae) or life forms (weeds). Some consider comparatively small circumscribed areas (for example, Canary Islands) and others vast land masses from Europe to Africa, North to South America or the Arctic and the Cool Temperate Zone. The emphasis in most of the twenty-five contributions, however, is evolutionary and taxonomic, while the geological and geographic aspects receive much less attention.

The topics considered include endemism, vicariousness, disjunction, connexions and correspondences between floras of different areas, the role of hybridization, the significance of apomixis and the migration of both wild and cultivated species.

On the methodological side, some attention is given to computer mapping and analysis, but few of the papers actually deal with the classification of species into floral elements on a geographical basis (chorology).

Although biology found its begin-

nings in a taxonomic and distributional approach to variation, detailed information on distribution in relation to evolution is sadly lacking. Admittedly, such information is accumulating, as witnessed by this symposium, but there can be few areas of biology in which there is less agreement and uniformity with regard to what data should be recorded, the manner of their presentation and, especially, guide-lines for their explanation. To some extent, this diffuseness reflects the recent preoccupation with molecular and cellular levels of organization. But the complexities of population structure and the magnitude of spatial and temporal organizations in nature present formidable problems of analysis and interpretation.

This valuable volume is an expression of the revival of interest in macrobiology which, hopefully, is now poised to proceed, if not as quickly, then as far as studies within the individual have done in recent years. Since its eminent contributors, burying old antagonisms and animosities, recognize the need for a combined attack utilizing the descriptive and analytical methods of many disciplines, this hope is more than pious. The only cause for concern lies in the departure from neo-Darwinian attitudes to evolution which commonly attends considerations of big biological problems, large areas, long periods of time and relatively unrelated organisms which one can neither cross nor, therefore, adequately classify.

K. R. LEWIS

Ovary Anatomy

Comparative Morphology of the Mammalian Ovary. By Harland W. Mossman and Kenneth L. Duke. Pp. xxviii+461. (University of Wisconsin: Madison, June 1973.) \$28.

THIS is a well-organized, comprehensive account of ovarian morphology containing much unpublished information collected by the authors. After chapters dealing with the gross anatomy and microscopic structure of the ovary an account is given of the development of the ovary. Chapter 4 describes changes in the ovary during the life cycle of a representative species (the red squirrel); the ovaries in this animal are small enough to permit a study of serial sections and the different cell types are easily distinguishable. However, these considerations also apply to the guinea-pig, which would be a better example to use since we know more about ovarian hormone production in this species. Chapter 6 is a readable account of comparative morphology, since most of the details of ovarian morphology in various taxonomic groups are presented in a series of synoptic tables and supplementary notes. In this way the general

trends become apparent and are not obscured by too much detail. In a field where there is much confusion about terminology the glossary defining the various terms which are used is extremely helpful. Those interested only in the human ovary will find chapter 5 and the chapter on "Features and Problems Associated with the Ovary", in which matters such as the origin of follicular fluid, follicular atresia, anovular and polyovular follicles are considered, both informative and interesting. This is an admirable book, profusely illustrated with diagrams and photographs, and will be invaluable to the comparative anatomist and endocrinologist.

K. FOTHERBY

Pollination

The Pollination of Flowers. By Michael Proctor and Peter Yeo. Pp. 418+200 photographs. (William Collins: London, April 1973.) £4.

It is inevitable that a book on this sadly neglected subject should contain much information on the structural modifications of flowers and insects associated with the evolution of pollination mechanisms. In these circumstances a book can easily become a catalogue of such relationships. The authors of this volume, however, have covered the morphological aspects of pollination in a brief yet systematic way, much information being summarized in tabular and diagrammatic form. Consequently much space is devoted to such problems as the respective roles of scent and sight in the behaviour patterns of the insect visitors. Of particular interest is the discussion of colour vision in bees and its significance in flower discrimination.

Undoubtedly the orchids represent the most highly adapted group of plants with respect to their pollination mechanisms, and a chapter is devoted to the British species. In the orchid genus *Ophrys*, the insect/flower relationship becomes little less than bizarre, the flower having evolved scent, visual and tactile modifications which release copulatory behaviour in the male *Halictus* solitary bee. The authors regard this type of modification as a form of parasitism upon the insect vectors via their behavioural responses.

As Darwin was well aware, the study of pollination mechanisms reveals how intense the pressures of natural selection can be, and in their final chapter the authors consider the development of pollination strategies in relation to the evolution of both flowering plants and insects.

The book is packed with interesting information and contains many of Michael Proctor's excellent photographs.

PETER D. MOORE

CORRESPONDENCE

Natural Units

SIR,—McWeeny¹ proposes the following abbreviations: as, aV and aT, meaning respectively atomic second, volt and tesla. It should be pointed out, however, that in the SI the prefix atto with the abbreviation a is already in use with the meaning 10^{-18} , that is $1 \text{ as} = 10^{-18} \text{ s}$, whereas according to McWeeny its meaning should be $24.19 \times 10^{-18} \text{ s}$. Clearly there might be confusion in this case. With the other units confusion is less likely, as they differ by a factor larger than 10^{10} . The prefix atto and its abbreviation have been recognized by various standardizing organizations and widely published², although admittedly they are so far not often met with in the literature.

There are two ways of avoiding this conflict. One is simply to use another prefix which when abbreviated would not conflict with SI. One possibility is sya, abbreviated s (or possibly sy). This prefix is derived from "système atomique", and hence would convey the same meaning as the original proposal. I hope the English-speaking readership will forgive the use of French in this case (cf. *Système International des Unités*). If there are serious arguments against this prefix, other non-conflicting alternatives may be found (for example wadi, abbreviated w, from "world of atomic dimensions").

Another, perhaps preferable, way of avoiding this conflict is to name a few more units after researchers of outstanding merit. Why not reintroduce the Rutherford (R or Ru) as the natural time unit, as the old meaning as a unit of radioactivity now may be considered obsolete? In case of doubt, the time unit might be called a Rutherford-second with the same abbreviation (whereas the radioactivity unit was abbreviated rd). Furthermore, Zeeman (Z) might be used in the sense of "atomic tesla" ($\hbar/ea_0^2$). For "atomic volt" perhaps no new name is needed, since it may so easily be written in the form Ha/e (hartree per electron, or per elementary charge). Here I have abbreviated hartree as Ha, considering that H is the accepted abbreviation of the SI unit henry.

These proposals would retain all the advantages of the system described by McWeeny, but avoid the conflict with

SI and its standardized abbreviations.

Yours faithfully,

BJØRN E. BØRDALEN

Central Institute for Industrial Research,
Oslo-Blindern, Norway

¹ McWeeny, R., *Nature*, **243**, 196 (1973).

² McGlashan, M. L., in *Kirk-Othmer Encyclopedia of Chemical Technology*, second ed. (edit. by Standen, A.), Suppl., 984 (Interscience, New York, London, Sydney, and Toronto, 1971).

Turning the Cheek

SIR,—While reading the interesting article by McManus and Humphrey¹ I was surprised to see that they do not mention the argument that right-handed painters, like right-handed writers, prefer to have the window or any other light source on their left. The subject will then be illuminated from his or her right hand side, and shadows on his left side will be more pronounced if he puts his right cheek forward than when his left cheek confronts the artist. Preference for a clearly delineated or a more gentle portrait might thus provide the answer to the authors' question, and also explain why the bias is strongest for female subjects.

Yours faithfully,

J. H. BANNIER

ZWO,

Postbus 2138,

's-Gravenhage

¹ *Nature*, **243**, 271 (1973).

John Milne

SIR,—We are researching the life and work of Professor John Milne (1850–1913), the father of seismology, who was born at Liverpool and died at Shide, Isle of Wight. We are anxious to obtain information about his life, work in Japan, photographic material, personal papers and relatives which will enable us to complete a comprehensive study of this famous but forgotten man. All material loaned will be carefully handled, documented, returned and acknowledged.

Yours faithfully,

LESLIE HERBERT-GUSTAR

PATRICK A. NOTT

Isle of Wight Technical College,
Newport, Isle of Wight PO30 5TA

Chromatography Priority

SIR,—Your correspondent (*Nature new Biol.*, **243**, 130; 1973) attributes the inception of affinity chromatography in enzyme purification to Cuatrecasas, Wilchek and Anfinsen (*Proc. natn. Acad. Sci. U.S.A.*, **61**, 636; 1968). May I note that these workers credit Lerman (*Proc. natn. Acad. Sci. U.S.A.*, **39**, 232; 1953) and Arsenis and McCormick (*J. biol. Chem.*, **239**, 3093; 1964) with prior attention to this technique?

Yours faithfully,

ROBERT F. B. DILLER

Baylor College of Medicine,
Texas Medical Center,
Houston, Texas 77025

Mental Illness

SIR,—Professor Szasz's reasoning (*Nature*, **242**, 305; 1973) is incontestable. However, the premise—that the doctor-patient relationship is a closed system—should not pass without comment.

The doctor, who is also a citizen, has obligations to society; and his philosophy should embrace not only the privacy of his patient but also the circumstances which gave rise to the patient's condition. Consequently the plausible argument, which is superficially sound, is philosophically worthless. It betrays an inability to accept or even acknowledge any whiff of humanism beyond the doctor's sworn commitment to his immediate patient.

The laboratory chemist no longer ignores industrial pollution; nor does the physicist radioactive fallout. The doctor too must face up to the wider implications of his special knowledge. The patient's disease should prompt the doctor's concern for public health; mutilation should evoke a desire to avert the carnage, on battlefield or highway; loss of self-confidence or "mental illness" should foster inquiry into stress; and incarceration should pose questions of ethics and economics.

Yours faithfully,

DOUGLAS H. RATCLIFFE

108 Waratah Avenue,
Dalkeith,
Western Australia 6009

Obituary

Lev Andreevich Artsimovich

LEV ANDREEVICH ARTSIMOVICH, the Soviet physicist, died on March 1, 1973.

Artsimovich, the son of a professor of statistics, was born on February 25, 1909, in Moscow. At nineteen years of age he graduated from the Byelorussian State University, in Minsk, and then began work at the Leningrad Physico-Technical Institute, where he was a student of A. F. Ioffe. From 1930 onwards, he taught at a number of higher educational and research institutes in Moscow and Leningrad, including Leningrad State University and the Leningrad Polytechnic Institute. From 1930 to 1948 he worked at the Physico-Technical Institute of the Academy of Sciences of the USSR, and between 1959 and 1963 he was Academic Secretary of the Department of Physico-Mathematical Sciences of the Academy. He was elected a corresponding member of the Academy in 1946 and a full member in 1953. In 1963 he became senior associate and Deputy Head of the Institute of Atomic Energy of the Academy, and was made a member of the Editorial Board of the *Doklady* of the Academy in 1960.

Artsimovich began his research with the study of X-ray optics and the problem of total X-ray reflexion. Between 1934 and 1935 he studied properties of (n_1n) and (n_1p) interactions. Work which he carried out in collaboration with I. V. Kurchatov and

others was among the first demonstration of the large magnitude of slow neutron capture cross section. In 1936, together with A. I. Alikhanov and A. I. Alikhanyan, he carried out an experiment which clearly confirmed the laws of conservation in electron-positron annihilation and refuted Shenkland's postulate that in this case the basic laws were violated.

During the end of the 1930s, Artsimovich was chiefly concerned with the interaction of fast electrons with matter. Then experimental data on bremsstrahlung and the angular distribution of scattered electrons differed from the accepted theory by two orders of magnitude. In an extensive series of experiments, Artsimovich succeeded in showing that the quantum-mechanical theory of the passage of fast electrons through matter agreed with experimental data to within the limits of experimental error.

During the war, Artsimovich worked on the theory of chromatic aberrations in electron-optical systems, carrying out considerable theoretical and experimental research on electron-optical converters. In 1945, together with L. Ya. Pomeranchuk, he carried out theoretical research on radiation losses in betatrons, which enabled the maximum energy achievable by betatron acceleration to be increased. He also formed part of a team which developed an electromagnetic method of isotope separation, being himself responsible for designing the optics of the ion source.

At the beginning of the 1950s, Artsimovich began studying the possibilities of controlled thermonuclear reactions, investigating high current pulse discharges in low pressure deuterium. During these experiments Artsimovich and his team succeeded in obtaining a highly ionized plasma of $1,000,000^\circ\text{C}$. In 1952 they discovered that a powerful pulse discharge in low pressure deuterium is a source of neutrons and short-wave X rays. Later, they demonstrated that the gas discharge plasma, compressed in the presence of a longitudinal magnetic field, possesses paramagnetic properties. They also showed that the neutrons originate not from thermonuclear reactions but from a specific acceleration process. Artsimovich was then increasingly associated with Soviet progress in thermonuclear research, and in 1958 he presented a report on Soviet research in this field to the Second World Congress on the Peaceful Uses of Atomic Energy. During the 1960s he played a leading part in research on Tokamaks, and achieved a long-duration stable plasma with a density of up to 10^{14} ions cm^{-3} and temperature of up to $5,000,000^\circ\text{C}$. For his work in this field, he was awarded a number of Soviet honours, including the Order of Lenin (four times), the order of the Red Banner of Labour (twice) and the Lenin Prize (1958). In 1965 he was awarded the Czechoslovak medal, and in 1966 was elected a foreign member of the Czechoslovak Academy of Sciences.

HOW TO BUY NATURE

The cost of one year's subscription to NATURE is:

	UK & elsewhere	US & Canada
Nature (Friday)	£16	£20
Nature & Nature Physical Science or Nature New Biology	£24	£33
Nature New Biology or Nature Physical Science	£10	£15
All three editions	£29.50	£44
Index (1972)	£1	£1.50

(Charges for delivery by air mail on application). Subscribers in North America may be able to claim a tax rebate against their NATURE subscription.

Editorial, Advertising and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF
Telex 262024

MACMILLAN JOURNALS LIMITED
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20004
Telephone Number: 202-737 2355. Telex 64280

Advertisement Department
MACMILLAN JOURNALS LIMITED
4 LITTLE ESSEX STREET, LONDON WC2R 3LF
Telephone Numbers: UK 01-836 6633. USA 202-737 2355

Subscription Department
MACMILLAN JOURNALS LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS RG21 2XS
Telephone Number: Basingstoke 29242

Classified advertisements
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone Number: 01-242 6264/01-405 4743
Telegrams: Textualist London WC2A 2ED
Registered as a newspaper at the Post Office